GLUTEN-FREE FRESH PASTA ENRICHED WITH BIOACTIVE COMPOUNDS

PATRÍCIA CATARINA DAS NEVES BORDALO BRANCO FRADINHO

SCIENTIFIC ADVISORS: PhD Anabela Cristina da Silva Naret Moreira Raymundo
PhD Isabel Maria Nunes de Sousa

THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE (PhD) IN
FOOD SCIENCE AND ENGINEERING

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2020

ACKNOWLEDGEMENTS

A PhD thesis is a personal victory because it is a work that goes on for several years and requires enormous resilience, a spirit of sacrifice and perseverance. Although is a very individual and lonely mission, this work was possible by the combined efforts of several institutions and individuals.

First of all, I would like to acknowledge the PhD scholarship awarded by the University of Lisbon (Ref. C0144M, BD2015) for 3 years, the support of the research unit LEAF - Linking Landscape, Environment, Agriculture and Food (UID/AGR/04129/2013) from Instituto Superior de Agronomia and the ERASMUS + grant (141/SMT/2017).

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Stones on the way? I keep them all. One day I'll build a castle.

ABSTRACT

Sustainability of the agri-food chain is on today's agenda, whether through the use of industrial by-products or the investigation of alternative sources of ingredients. The increasing number of individuals with gluten-related diseases, but also consumers following a gluten-free diet are the drivers for the continually growing number of gluten-free products launched in the market. However, in addition to their still limited commercial availability and high price compared to their wheat counterparts, gluten-free products show nutritional deficiencies.

Following the current consumer trends for healthy foods with underlying sustainable principles (ingredients and processes), this thesis aimed the development of a gluten-free fresh pasta based on rice flour from broken grains and *Psyllium* husk, enriched with *Arthrospira platensis, Laminaria ochroleuca* and potato peel.

Firstly, the gelatinisation (5 – 26%) of rice flour from broken rice (rice processing industry by-product) was performed to assess the optimum conditions for producing gluten-free pasta. Afterwards, the pasta formulation (50% gelatinised rice flour/rice flour) was successfully optimized, and its structure reinforced with 4% *Psyllium* husk gel. This gluten-free pasta was then enriched with bioactive compound sources (*Arthrospira platensis, Laminaria ochroleuca* and potato peel) incorporated as lyophilised biomass, gel or liquid extract obtained by subcritical water extraction (autohydrolysis), an eco-friendly technology.

The pastas developed were characterized in terms of cooking quality parameters, texture, colour, rheology, nutritional composition, antioxidant activity, *in vitro* digestibility and sensory analysis. It was found that autohydrolysis was a suitable technology for the valorisation of distinct materials, namely marine and industrial.

Pastas with *Laminaria ochroleuca* provided a high mineral and fibre contents, and low-fat content, so that could bear nutritional claims. On the other hand, *Arthrospira platensis* incorporation proved to have a positive effect on the pasta antioxidant activity and overall sensory analysis without affecting the *in vitro* digestibility. Pasta with potato peel extract presented a high total phenolic content and antioxidant activity. All formulations presented good overall cooking quality and texture and rheology properties.

Keywords: sustainability, gluten-free pasta, mechanical properties, by-products, healthy food

RESUMO

A sustentabilidade da cadeia agroalimentar é uma das tendências do setor, seja pela valorização de subprodutos industriais quer através da utilização de fontes alternativas e / ou de ingredientes pouco explorados. O número crescente de indivíduos com doenças relacionadas com o glúten, mas também consumidores que optam por seguir uma dieta sem glúten, é responsável pelo contínuo lançamento de produtos sem glúten no mercado. No entanto, além da sua disponibilidade comercial ainda limitada e elevado preço em comparação com produtos semelhantes com trigo, os produtos sem glúten apresentam carências nutricionais.

Seguindo as tendências atuais do setor agroalimentar e respondendo à crescente procura dos consumidores por alimentos saudáveis desenvolvidos de forma sustentável em termos de ingredientes e processos, esta tese visou o desenvolvimento de uma massa alimentícia fresca sem glúten elaborada com farinha de trinca de arroz e casca de *Psyllium*, enriquecida com *Arthrospira platensis*, *Laminaria ochroleuca* e casca de batata.

Numa primeira fase procedeu-se à gelatinização (5 - 26%) de farinha de trinca de arroz (subproduto da indústria de descasque e polimento de arroz) para avaliar as condições ótimas para o desenvolvimento de massas alimentícias sem glúten. Posteriormente, a formulação da massa (50% de farinha de arroz gelatinizada / 50% de farinha de arroz) foi otimizada através da utilização de 4% de gel de casca de *Psyllium* tendo-se obtido um reforço na sua estrutura. A massa desenvolvida foi então enriquecida com fontes de compostos bioativos (*Arthrospira platensis*, *Laminaria ochroleuca* e casca de batata), incorporadas como biomassa liofilizada, gel ou extrato líquido obtido por autohidrólise, uma tecnologia ambientalmente sustentável.

As massas desenvolvidas foram caracterizadas em termos de qualidade da cozedura, textura, cor, reologia, composição nutricional, atividade antioxidante, digestibilidade in vitro e análise sensorial. Demonstrou-se que a autohidrólise é uma tecnologia adequada para a valorização de materiais de diversas origens, nomeadamente marinha e industrial.

As massas com *L. ochroleuca* apresentaram elevados teores de minerais e fibras, e baixo teor de lípidos, podendo ser alvo de alegações nutricionais. Por outro lado, a incorporação de *A. platensis* evidenciou ter um efeito positivo na atividade antioxidante da massa e nas suas características sensoriais, sem afetar negativamente a digestibilidade in vitro. As massas com extrato de casca de batata apresentaram elevado teor de compostos fenólicos totais e atividade antioxidante. Todas as formulações evidenciaram boa qualidade de cozedura e propriedades de textura e reologia.

Palavras-chave: sustentabilidade, massa sem glúten, propriedades mecânicas, subprodutos, alimentos saudáveis

RESUMO ALARGADO

As escolhas alimentares dos consumidores dependem de vários fatores, nomeadamente do preço alimentos, de tradições culturais e religiosas, de fatores geográficos e ambientais, da disponibilidade de alimentos e de preferências individuais. O consumidor é, por isso, o grande impulsionador do mercado, ditando as tendências de consumo. À medida que os mercados se tornam mais exigentes e seletivos, as empresas são forçadas a adaptar-se para atender às novas necessidades dos clientes, adotando para isso estratégias muito diversificadas.

A sustentabilidade da cadeia agroalimentar é uma das importantes tendências do setor, podendo ser alcançadas quer pela valorização de subprodutos industriais, quer através da utilização de fontes alimentares alternativas e / ou de ingredientes pouco explorados.

O desenvolvimento de produtos direcionados para mercados com necessidades alimentares especiais, é outra das tendências relevantes da indústria alimentar, dos últimos anos. O número crescente de indivíduos com doenças relacionadas com o glúten, mas também consumidores que optam por seguir uma dieta sem glúten, é responsável pelo contínuo lançamento de produtos sem glúten no mercado. No entanto, além da sua disponibilidade comercial ainda ser limitada e apresentarem elevado preço, em comparação com produtos semelhantes com trigo, os produtos sem glúten apresentam geralmente associadas carências nutricionais, nomeadamente de vitaminas (B12, D) e minerais (ferro, cálcio, zinco), além de elevados teores de lípidos e calorias.

Seguindo as tendências atuais do setor agroalimentar e respondendo à crescente procura dos consumidores por alimentos saudáveis desenvolvidos de forma sustentável em termos de ingredientes e processos, a presente dissertação visou o desenvolvimento de massas alimentícias frescas sem glúten elaborada com farinha de trinca de arroz e casca de *Psyllium*, enriquecida com ingredientes alimentares pouco explorados: *Arthrospira platensis* e *Laminaria ochroleuca* e subprodutos da industria alimentar: extratos de casca de batata.

Numa primeira fase procedeu-se à gelatinização (5 – 26 %) de farinha de trinca de arroz (subproduto da indústria de descasque e polimento de arroz) para avaliar as condições ótimas para o desenvolvimento de massas alimentícias sem glúten. Foram utilizadas duas variedades de arroz (Guiana e Ariete, das subespécies *Indica* e *Japonica*, respetivamente). Através de ensaios físico-químicos, reológicos e de textura verificou-se que ambas as variedades de arroz podem ser utilizadas para o desenvolvimento de produtos sem glúten que requerem amido pré-gelatinizado, como é o caso de massas isentas de glúten. No entanto, por constrangimentos do mercado em termos de disponibilidade de farinha de arroz, procedeu-se à otimização da massa utilizado farinha de arroz comercial constituída por uma mistura de arroz *Indica* e *Japonica*. Estudou-se o efeito da farinha de arroz (10–25 %) e da proporção gel de arroz / farinha de arroz (40:60, 50:50, 60:40) nas propriedades físicas e na qualidade da cozedura da massa, tendo-se concluído que a formulação com 50 % de farinha

de arroz gelatinizada (elaborada com 20 % de farinha de arroz) / 50 % de farinha de arroz) foi a que apresentou maior qualidade em termos de cozedura e propriedades de textura. Verificou-se assim a possibilidade de utilizar os géis de *Psyllium* desenvolvidos na preparação de massas isentas de glúten, sendo este um processo alternativo aos processos tradicionais que envolvem etapas prévias de pré-gelatinização do amido. Esse processo é mais complexo e demorado, apresentando um elevado gasto de energia.

Posteriormente, a formulação da massa foi otimizada através da utilização de casca de *Psyllium,* a qual apresenta a capacidade de formar gel à temperatura ambiente. Neste estudo foi avaliado o efeito do tamanho de partícula do *Psyllium*, a temperatura de processamento e a concentração de gel de *Psyllium* na qualidade de massas sem glúten. Foram determinados os parâmetros de qualidade de cozedura, propriedades de textura, a composição nutricional, a capacidade antioxidante e a digestibilidade *in vitro* das massas. A massa com incorporação de 4 % de casca de *Psyllium* (160 - 315 µm de tamanho de partícula, processado termicamente a 40ºC) apresentou uma maior qualidade da cozedura, tendo-se igualmente obtido um reforço na sua estrutura.

De acordo com a procura por alimentos saudáveis e para colmatar algumas carências nutricionais da população celíaca foram projetados três estudos, que tiveram como objetivo global enriquecer a massa sem glúten desenvolvida com compostos bioativos. Para tal recorreu-se à incorporação de *Laminaria ochroleuca* e de seus extratos, biomassa de *Arthrospira platensis* e extratos de casca de batata.

A alga castanha *Laminaria ochroleuca* mostrou potencial para ser valorizada integralmente ou através dos seus extratos líquidos obtidos por autohidrólise, uma tecnologia ambientalmente sustentável. A aplicação desta alga em massas isentas de glúten conduziu a um aumento do teor de fibras e minerais, e baixo teor de lípidos, podendo ser alvo de alegações nutricionais (*fonte de fibra* e *sem gordura*). Além disso, a massa isenta de glúten apresenta propriedades mecânicas e sensoriais com interesse de exploração comercial futura. Esta fase do trabalho foi realizada em parceria com a Universidade de Vigo.

O desenvolvimento de massas isentas de glúten constitui um desafio em termos tecnológicos. Neste sentido, a incorporação de *Arthrospira platensis* (vulgarmente conhecida por Spirulina) constitui uma mais valia para reforçar a criação de estrutura em matrizes sem glúten, resultante do seu elevado teor proteico, além dos benefícios nutricionais associados. Este estudo, realizado em parceria com a Universidade de Florença, avaliou o impacto da incorporação de *Arthrospira platensis* de duas origens (F&M-C256 e comercial) na qualidade tecnológica e valor nutricional de massas alimentícias isentas de glúten. As massas desenvolvidas foram caracterizadas em termos de qualidade da cozedura, textura, cor, reologia, composição nutricional, atividade antioxidante, digestibilidade *in vitro* e análise sensorial. A incorporação desta cianobactéria evidenciou ter um efeito positivo na atividade

antioxidante da massa e nas suas características sensoriais, sem afetar negativamente a digestibilidade *in vitro*.

Os processos de colheita e armazenamento de batatas são responsáveis pela produção de grandes quantidades de resíduos (até 30%), os quais são encaminhados principalmente para alimentação animal. Além disso, aliado ao facto de a batata ser a principal fonte de amido, e ao aumento de produtos processados de batata, são originadas grandes quantidades de subprodutos (maioritariamente cascas), que constituem uma excelente fonte de fibras, minerais e antioxidantes, podendo ser recuperadas e usadas na produção de produtos de valor acrescentado, como alimentos sem glúten destinados à população celíaca. A recuperação e valorização destes subprodutos constitui simultaneamente uma necessidade e uma mais-valia para a indústria. Seguindo as tendências da utilização de processos industriais ambientalmente sustentáveis, recorreu-se à extração dos componentes bioativos da casca de batata pelo processo de autohidrólise, usando a água como o único agente de extração. Trata-se de um processo compatível com alimentos, apresenta custos inferiores aos processos tradicionais e é "amiga" do ambiente, podendo ser realizada usando alta temperatura e pressão para aumentar a eficiência das extrações. Neste estudo foram usadas batatas de três variedades de batata (Kennebec, Neiker e Agria), tendo as condições de extração sido otimizadas com a variedade Kennebec, por ser a mais abundante, num reator pressurizado entre 160^{o}C e 220^{o}C. A fração líquida obtida foi caracterizada em termos de compostos fenólicos totais e atividade antioxidante. As condições ideais de autohidrólise (220^{o}C, 7,5 atm) foram aplicadas a todas as outras variedades de batata, e a variedade Agria foi selecionada para aplicação em massas isentas de glúten. Foi avaliado o impacto do extrato líquido de autohidrólise de casca de batata na composição nutricional, qualidade de cozedura, propriedades de textura, e reologia das massas (cruas e cozidas), tendo-se concluído que as massas isentas de glúten enriquecidas apresentaram propriedades tecnológicas interessantes, aliadas a uma cor atrativa, ao aumento do teor de compostos fenólicos e a uma maior atividade antioxidante. Concluiu-se que a tecnologia de autohidrólise é adequada para a recuperação eficiente dos compostos com capacidade antioxidante das cascas de batata de descarte, permitindo a sua valorização.

Assim, foi possível desenvolver massas alimentícias sem glúten, com recurso a um processo tecnológico inovador, baseado na utilização de géis de *Psyllium* e testaram-se alternativas viáveis de enriquecimento nutricional dessas massas, baseadas na utilização de recursos sustentáveis: micro e macroalgas e subprodutos da indústria alimentar.

Palavras-chave: sustentabilidade, massa sem glúten, propriedades mecânicas, subprodutos, alimentos saudáveis

THESIS OUTLINE

The thesis was divided into six major chapters, each one described briefly below. Figure A represents a general diagram of the entire PhD study. The schematic summary of each chapter, highlighting the specific objectives, methods and outcomes is presented at the beginning of the respective chapter.

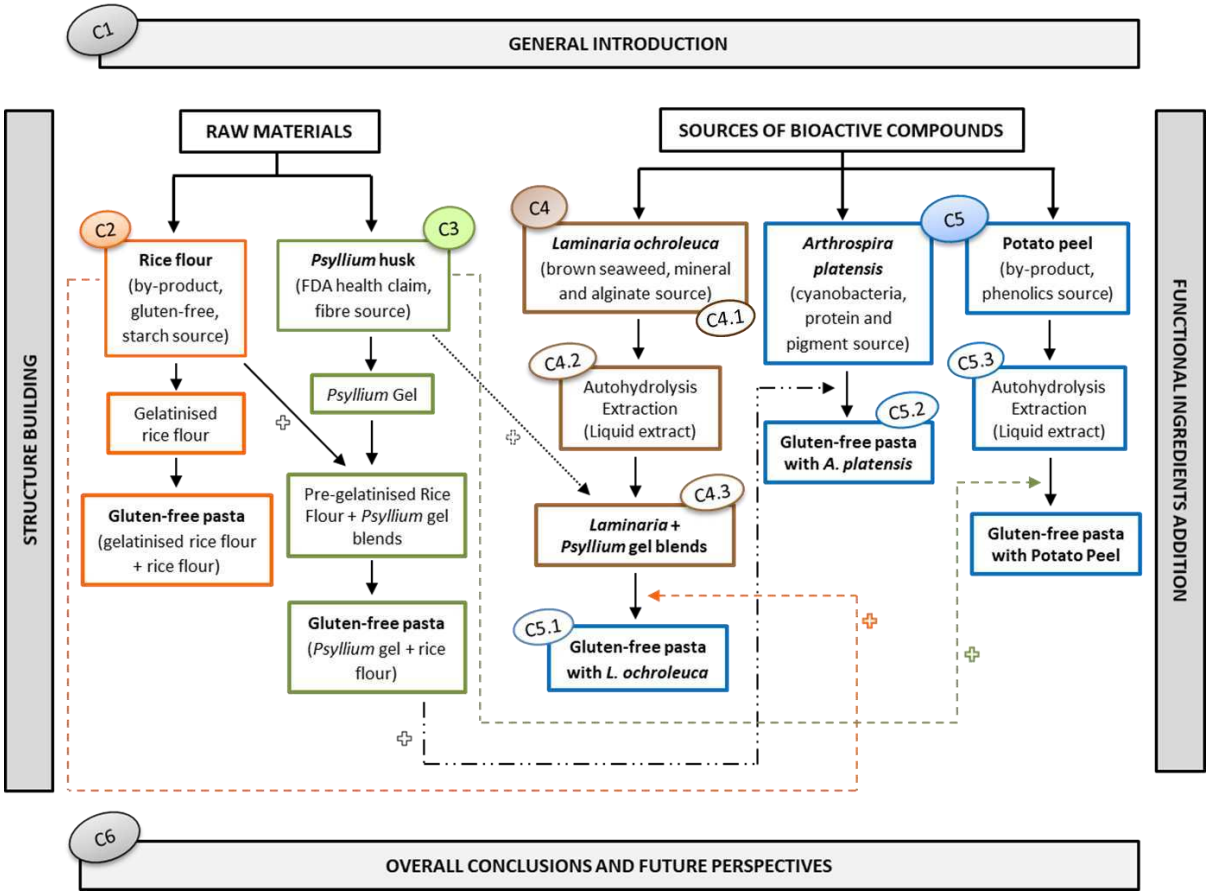

Figure A. Conceptual diagram of the study. Each chapter and subchapter (e.g. C1, C6.1) is represented by a different colour.

Chapter 1. General introduction. This chapter (C1) contains the contextualization of the study, highlighting the current food trends related to the study, i.e. empowered consumer, sustainability and functional foods. The main objectives of this work are also presented here.

As this thesis is the compilation of the scientific work published (or to be), an extensive literature review chapter is not included since this is comprised in the introduction sections of each publication.

Chapter 2. Functional and thermorheological properties of rice flour gels for gluten-free pasta applications. This chapter (C2) deals with the evaluation of the chemical and hydration properties of rice flour from broken kernels, a by-product from the rice industry. The impact of the rice variety on the texture and viscoelasticity of rice flour gels was assessed, to evaluate the potential of using this by-product in the development of gluten-free pasta.

Chapter 3. *Psyllium* husk gel to reinforce structure of gluten-free pasta. In this chapter (C3), the gluten-free pasta formulation developed in chapter 2 was optimized taking advantage of *Psyllium*'s husk gelling ability. A fundamental rheological approach was adopted in order to assess the optimal processing conditions (size particle, temperature and concentration) of *Psyllium* gels prior to dough incorporation, to develop fresh pasta. Gluten-free pasta samples were characterized in terms of chemical composition, colour stability upon cooking, texture rheological behaviour, antioxidant capacity and *in vitro* digestibility.

Chapter 4. Study and processing of *Laminaria ochroleuca* for gel applications. This chapter (C4) is focused on the recovery and valorisation of the edible brown alga *Laminaria ochroleuca* for food gel applications. It comprises three publications: a mini review (C4.1) on the use of by-products from the marine, agricultural and forestry resources for the development of novel hydrogels with functional properties, and two studies regarding the application of a green technology to process and recover bioactive fractions of this macroalga (C4.2.) and its combination with *Psyllium* to develop food gel applications (C4.3.).

This work was performed in collaboration with the University of Vigo (*Campus* Ourense), in the research group of Professor Herminia Domínguez whose expertise on the use of green efficient technologies for the integral utilization of natural renewable resources such as seaweeds for food and non-food applications. Acknowledging an ERASMUS+ grant for joining her team for 4 months.

Chapter 5. Gluten-free pasta with bioactive compounds. This chapter (C5) comprehends the incorporation of new sources of ingredients (*Arthrospira platensis* and *Laminaria ochroleuca*) and potato peel (industry by-product) in gluten-free pasta, using the formulation optimized in chapter 3 as control. It comprises three incorporation studies: one using the knowledge achieved in previous chapter, to enhance the technological and nutritional properties of gluten-free pasta (C5.1); other dealing with the effect of *Arthrospira platensis* incorporation on the rheological and bioactive properties of gluten-free pasta (C5.2); and finally a study where the autohydrolysis processing technology used in chapter 4 was applied to potato peel to obtain a liquid extract for further incorporation in gluten-free pasta (C5.3).

In this chapter, collaboration established with the University of Vigo (*Campus* Ourense) continued, and another one started with the University of Florence, namely with the research group coordinated by Professor Mario R. Tredici whose expertise on the design of novel photobioreactors and microalgae exploitation for food and feed, nutraceuticals, biofuels and CO_2 biofixation.

Chapter 6. Overall conclusions and future perspectives. In this chapter (C6) the major conclusions of the thesis are highlighted, and future perspectives of this work are also discussed.

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Fradinho, P., Sousa, I., Raymundo, A. (2019). Functional and Thermorheological Properties of Rice Flour Gels for Gluten-Free Pasta Applications. *International Journal of Food Science & Technology*, 54, 1109-1120. https://doi.org/10.1111/ijfs.14001 (Impact Factor: 2.281, Q2)

Chapter 3

- ***Research Paper 2***

Fradinho, P., Soares, R., Niccolai, A., Sousa, I., Raymundo, A. (2020). *Psyllium* husk gel to reinforce structure of gluten-free pasta? *LWT – Food Science and Technology,* 131, 109787. https://doi.org/10.1016/j.lwt.2020.109787 (Impact Factor: 3.714, Q1)

Chapter 4

- ***Technical Review***

Torres, M.D., Fradinho, P., Domínguez, H. (2018). New life for marine, agricultural and forestry by-products - personal care hydrogels with functional features. *H&PCToday*, 13(6): 7-11. https://www.teknoscienze.com/wp-content/uploads/2018/11/Torres.pdf

- ***Research Paper 3***

Fradinho, P., Flórez-Fernández, N., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2020). Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive

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Fradinho, P., Sousa, I., Raymundo, A. (2019). Functional and Thermorheological Properties of Rice Flour Gels for Gluten-Free Pasta Applications. *International Journal of Food Science & Technology*, 54, 1109-1120. https://doi.org/10.1111/ijfs.14001 (Impact Factor: 2.281, Q2)

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- ***Research Paper 4***

Fradinho, P., Raymundo, A., Sousa, I., Domínguez, H., Torres Pérez, M.D. (2019). *Psyllium* and *Laminaria* partnership – an overview of possible food gel applications. *Applied Sciences*, 9(20), 4356. https://doi.org/10.3390/app9204356 (Impact Factor: 2.217, Q2)

Chapter 5

- ***Research Paper 5***

Fradinho, P., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2019). Edible brown seaweed in gluten-free pasta: technological and nutritional evaluation. *Foods*, 8(12), 622. https://doi.org/10.3390/foods8120622 (Impact Factor: 3.011, Q2)

- ***Research Paper 6***

Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A. (2020). Effect of *Arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research*, 45, 101743. https://doi.org/10.1016/j.algal.2019.101743 (Impact Factor: 3.723, Q1)

- ***Research Paper 7***

Fradinho, P., Oliveira, A., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2020). Improving the nutritional performance of gluten-free pasta with potato peel autohydrolysis extract. *Innovative Food Science and Emerging Technologies,* 63, 102374. https://doi.org/10.1016/j.ifset.2020.102374 (Impact Factor: 4.085, Q1)

LIST OF PUBLICATIONS

The resulting publications of this work are collected in the following list. From this thesis seven articles have been published in international scientific journals indexed in ISI-Web of Science, and a mini review published in a technical journal.

The work presented on this thesis has also been presented in several national and international scientific conferences, either as oral (four) or poster (four) communication, one of which has received a best poster award in the respective scientific meeting.

At the end of this list, several publications (two research papers, one poster and one oral communication) produced within the thesis framework are presented. These studies intended to gain insight on the characterization of some of the raw materials used in the thesis, and the optimization of procedures associated with the thesis, but they were not included in the main core as the candidate is not the first author.

A. PUBLICATIONS IN INTERNATIONAL SCIENTIFIC JOURNALS WITH PEER REVIEW

- ***Publications ISI -Web of Science***

Fradinho, P., Sousa, I., Raymundo, A. (2019). Functional and Thermorheological Properties of Rice Flour Gels for Gluten-Free Pasta Applications. *International Journal of Food Science & Technology*, 54, 1109-1120. https://doi.org/10.1111/ijfs.14001 (Impact Factor: 2.281, Q2)

Fradinho, P., Raymundo, A., Sousa, I., Domínguez, H., Torres, M.D. (2019). *Psyllium* and *Laminaria* partnership – an overview of possible food gel applications. *Applied Sciences*, 9(20), 4356. https://doi.org/10.3390/app9204356 (Impact Factor: 2.217, Q2)

Fradinho, P., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2019). Edible brown seaweed in gluten-free pasta: technological and nutritional evaluation. *Foods*, 8(12), 622. https://doi.org/10.3390/foods8120622 (Impact Factor: 3.011, Q2)

Fradinho, P., Flórez-Fernández, N., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2020). Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties. *Journal of Applied Phycology,* 32, 1455-1465. https://doi.org/10.1007/s10811-019-01958-8 (Impact Factor: 2.635, Q1)

Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A. (2020). Effect of *Arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research,* 45, 101743. https://doi.org/10.1016/j.algal.2019.101743 (Impact Factor: 3.723, Q1)

Fradinho, P., Soares, R., Niccolai, A., Sousa, I., Raymundo, A. (2020). *Psyllium* husk gel to reinforce structure in gluten-free pasta? *LWT – Food Science and Technology,* 131, 109787. https://doi.org/10.1016/j.lwt.2020.109787 (Impact Factor: 3.714, Q1)

Fradinho, P., Oliveira, A., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2020). Improving the nutritional performance of gluten-free pasta with potato peel autohydrolysis extract. *Innovative Food Science and Emerging Technologies*, 63, 102374. https://doi.org/10.1016/j.ifset.2020.102374 (Impact Factor: 4.085, Q1)

- ***Technical review***

Torres, M.D.; Fradinho, P.; Domínguez, H. (2018). New life for marine, agricultural and forestry by-products - personal care hydrogels with functional features. *H&PCToday*, 13(6): 7-11. https://www.teknoscienze.com/wp-content/uploads/2018/11/Torres.pdf

B. PRESENTATIONS IN SCIENTIFIC MEETINGS

- ***Oral communications***

Fradinho, P., Torres, M.D., Flórez-Fernández, N., Sousa, I., Raymundo, A., Domínguez, H. (2018). *Laminaria ochroleuca* brown seaweed and its autohydrolysis extracts features. Book of Abstracts WEFTA2018. Pp. 63. (Lisbon, Portugal, 15-18 October). Oral Communication O42.

Fradinho, P., Sousa, I., Raymundo, A. (2019). The role of *Psyllium* gels on the structuring of gluten-free fresh pasta – a rheological approach. AERC2019, Slovenia, 8-11 April. Oral Communication FP11.

Fradinho, P., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2019). *Psyllium-Laminaria gels* for food and non-food applications. Book of Abstracts of IBEREO 2019. Pp.50 (Porto, Portugal, 4-6 September). Oral Communication FCPP1.

Fradinho, P., Oliveira, A., Domínguez, H., Torres, M.D., Sousa, I., Raymundo, A. (2019). *Biorefinery concept applied to kombu for enriching gluten-free pasta*. Book of Abstracts of AlgaEurope 2019. Pp.163-16450 (Paris, France, 3-5 December). Oral Communication 11.3.

- ***Poster communications***

Fradinho, P.; Sousa, I.; Raymundo, A. (2017). Psyllium husk' rheological properties for application in gluten-free pasta. Book of Abstracts of the IBEREO2017, Valencia, Spain. Pg 103. Poster FR-P16. **Best Poster Award** promoted by EPL (Europhysics Letters).

Fradinho, P., Niccolai, A., Soares, R., Sousa, I., Rodolfi, L., Biondi, N., Tredici, M.R., Raymundo, A. (2018). Microalgae: an unexplored source to develop functional gluten-free pasta. Book of Abstracts of FBHC2018. Pp 260. (Lisbon, Portugal, 26-28 September). Poster 148.

Fradinho, P., Soares, R., Niccolai, A., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A. (2018). Efeito da Composição Nutricional de *Arthrospira platensis* na Qualidade de Massas Isentas de Glúten. Livro de Resumos do 14ºEQA. Pp.68. (Viana do Castelo, Portugal, 6-9 Novembro). Poster CP012.

Fradinho, P., Sousa, I., Raymundo, A. (2019). Gluten-Free Fresh Pasta Enriched with Bioactive Compounds. Encontro com a Ciência e Tecnologia em Portugal – Ciência'2019. (Lisboa, Portugal, 8-10 Julho). Poster.

C. OTHER PUBLICATIONS PERFORMED WITHIN THE THESIS FRAMEWORK

Batista, A.P., Niccolai, A., Fradinho, P., Fragoso, S., Sousa, I., Raymundo, A., Rodolfi, L., Biondi, N., Tredici, M.R. (2016). Microalgae biscuits – sensory, physical and chemical properties, antioxidant activity and *in-vitro* digestibility. AlgaEurope2016, Madrid, Spain. Oral Communication.

Morais, A., Fradinho, P., Sousa, I., Raymundo, A. (2017). Design of different food materials based on the gelling ability of rice flour. 11th Annual European Rheology Conference, AERC2017, Denmark. Poster PO47.

Batista, A.P., Niccolai, A., Fradinho, P., Fragoso, S., Bursic, I., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A. (2017). Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. *Algal Research*, 26, 161-171. https://doi.org/10.1016/j.algal.2017.07.017 (Impact Factor: 3.723, Q1)

Valdez-Meza, E.E., Raymundo, A., Figueroa-Salcido, O.G., Ramírez-Torres, G.I., Fradinho, P., Oliveira, S., Sousa, I., Suárez-Jiménez, M., Cárdenas-Torres, F.I., Islas-Rubio, A.R., Rodríguez-Olibarría, G., Ontiveros, N., Cabrera-Chávez, F. (2019). Pasta Enrichment with an Amaranth Hydrolyzate Affects the Overall Acceptability while Maintain Antihypertensive Properties. *Foods,* 8(8), 282. https://doi.org/10.3390/foods8080282 (Impact Factor: 3.011, Q2)

Chapter 1. General introduction

1.1. Trends and Innovations in the Food Sector

People's food choices depend on various factors such as food prices, socio-economic status, level of education, cultural and religious traditions, geographical, environmental factors, availability and individual preferences (Bowman, 2006; Pala et al., 2006; Chauke & Duh, 2019; Eufic, 2020).

As consumers become more demanding, the global market is forced to adapt to meet changing customer needs. Each year, consulting and market firms (e.g. Deloitte, Euromonitor International, Mintel), research organisations (e.g. CSIRO, SITRA) and other professionals, publish their forecasts for the market trends. As not all trends are directly related to food, a selection was made and the common elements between the various publications were combined. Next, some current trends that relate to the present thesis, are briefly described.

1.1.1. Empowered Consumers

Consumer preferences are constantly shifting. Currently, consumers want fresh food fast, in convenient formats and with recognizable ingredients, and more transparency from the food industry (Angus & Westbrook, 2020; Renner et al., 2019; Williams, 2019). **Trust** is the prime factor influencing consumers food choices globally (65%), followed by convenience (time and financial constraints) (63%) and health impact (62%) (Hendry, 2019). A way to generate trust is to listen to the consumer's needs. Consumers increasingly claim for greater access to information about the origin of their food, its ingredients, and their production and market processes.

Clean label products can be understood as foods exhibiting an ingredient list which is characterised by being "short, simple, no artificial ingredients, not 'chemical sounding', with 'kitchen cupboard ingredients' that are expected and familiar" (Asioli et al., 2017). Consequently, food producers respond by adapting their ingredient lists to move closer to the idea of *clean label* foods (Aschemann-Witzel et al., 2019), helping to build trust in the brand. As an example, in terms of the protein plant-based food, companies should specify the exact plant source of the protein in the ingredient list, especially when targeting consumers interested in vegetarian alternatives, improving consumer perception (Aschemann-Witzel & Peschel, 2019).

In this sense, food labelling assumes a major role in the communication with the consumer, that perceives nutrition labels as means to prevent fraud and assuring the quality and safety of the food product (Gracia et al., 2016). There is a positive association between nutrition label use and healthy food choices (Ni Mhurchu et al., 2018), with consumers with higher nutrition knowledge being more likely to use the nutrition facts panel when shopping (Barreiro-Hurlé et al., 2010). In the European food market, food labelling is mandatory to inform customers, providing trust on different quality characteristics of the food products (Gracia et al., 2016). Therefore, nutrition labelling may be an

effective approach to empower consumers in choosing healthier products, being front-of-package and interpretive labels (e.g. traffic light) the most effective (Cecchini & Warin, 2016; Gorski Findling et al., 2018).

In line with **transparency** and cleaner labels, the natural appeal is another current request of the consumers, with 80% of consumers actively seeking healthier versions of the food they purchase and 77% avoiding food with preservatives and chemicals (Renner et al., 2019). Naturalness in food is associated to traditional and *authentic* processing, leading to assumptions about favourable health effects (Amos et al., 2014). Consumer demands for natural and less processed food and drink are forcing companies to remove artificial ingredients. The use of natural flavours and colours in food and beverage market is increasing globally, with a 6 to 9% year growth, with Bakery and Confectionery as the top categories of global product launches (Food Ingredients First, 2019).

Natural claims are also evolving to provide greater clarity about the benefits of these products. Consumers increasingly demand for total transparency from the food and drink companies, including replacing vague claims like *all natural* by specific claims such as *GMO*[1]*-free* or *preservative-free* (Mintel, 2018).

The so called **"Free-from" foods** have slowly become mainstream health-food products, appealing to all consumers. Therefore, 'free-from' food market will continue to expand with an expected global rise of 9.5% rate until 2025, led by the United States, the United Kingdom, Italy, Germany, France, Spain, and Canada in the gluten-free category (Mordor Intelligence, 2020). This market is segmented by type (e.g. gluten free, dairy free, allergen free); product (e.g. bakery and confectionery, dairy free foods, snacks, beverages); distribution channel (e.g. supermarkets/hypermarkets, online retail stores, convenience stores). In Europe, the free-from food market is made up predominantly of gluten-free and dairy-free products. The gluten-free market is also increasingly accessible to the general consumer, with gluten-free per capita forecasts predicting a strong uptake in gluten-free purchases, particularly in Finland (27.5 to 34.5 US Dollars from 2015 to 2020) (Statista, 2018).

The development of **gluten-free foods** is on today's agenda, with the steadily increase of the gluten-free products market, due to the growing number of individuals diagnosed with some type of gluten sensitivity, but also by food choices made by many non-celiac consumers, since around 38% of consumers are avoiding or limiting gluten-free foods (Hendry, 2019). However, gluten-free foods are nutritionally unbalanced in terms of lipids, fibres, minerals and vitamins, which is especially critical for celiac patients or those with some type of sensitivity to wheat / gluten (Thompson et al., 2005; Kinsey, 2008; Missbach et al., 2015). Is no surprise that this population group is more prone to make their own foods at home, either by low availability of products destined to their needs, more expensive than wheat standards (Singh & Whelan, 2011; Allen & Orfila, 2018) either by distrust in the available ones

[1] Genetically Modified Organism

due to presence of gluten (Bustamante, 2017). This is a clear opportunity for the food industry to developed gluten-free products which are safe, nutritionally balanced and sensory appealing.

The free-from foods market is also a highly fragmented and competitive one, with the presence of various prominent and local players, where the major players hold less than 30% of the market (Mordor Intelligence, 2020). This is supported by the growing distrust in multinational food companies, and the return to traditions and roots (Batt, 2018). Today, consumers are moving away from globalisation and over-consumption and favouring higher-quality products while supporting local communities and independent traders. An important driver is environmental concerns as consumers associate local with being ecologically responsible (Angus & Westbrook, 2020). In addition, more than half of consumers believe that local brands and products are more authentic than products from other countries. **Producing locally**, companies can shorten delivery times, in line with manufacturers' economically sustainable ideals (Angus & Westbrook, 2020).

The disruption of traditional consumption occasions is changing consumers eating habits and behaviours. Today's busy lifestyles demand for more convenient items, offering retailers a competitive opportunity (NRF, 2020). Multinationals are becoming more sophisticated in shaping their products to local tastes and preferences without losing their core brand identity. This trend led to an increase in *Ready-to-eat* food and in the *Delivery-friendly* food sector, accounting for more than 60% of restaurant traffic, which explains the growth of takeout, delivery, drive-thru, and food truck vending options (NRA, 2018). With increased restaurant meal consumption happening outside of the restaurant comes a need for packaging materials that assures food quality. Linked with consumer' sustainability concerns but also with local legislation and regulations, many brands are meeting the challenge of reducing packaging, increasing recyclability and offering eco-friendly packaging (Seifer, 2019).

For short, the consumer demands for more safe, healthy and nutritious food that has been produced in a more sustainable and ethical manner. Therefore, this empowered consumer can be considered the drive for the other food trends.

1.1.2. Sustainability

With rising human population and increasing *per capita* wealth, the demand for food, feed, and other agricultural products is expected to increase in the future. Therefore, agricultural production (of all crop and livestock products) should increase 60% by 2050 (Alexandratos & Bruinsma, 2012).The world has produced more calories *per capita* over the last 50 years than ever before, but undernourishment and micronutrient deficiencies remain in many parts of the world, and increasing obesity is posing new human health challenges (Ramankutty et al., 2018). Around a third of all food produced is lost at some point of the food supply chain: during on-farm to post-harvest handling and storage, processing and packaging, distribution and in the market, and lastly by the consumer (FAO, 2011).

The last few years sustainability issues have risen in consumer concerns, especially amongst the new generations which hope innovation processes will reduce the environmental impact (Bollani, 2019). Currently, around 87% of consumers are worried about climate change and 54% think they can make a positive contribution in the world with their purchases (Angus & Westbrook, 2020). As environmental awareness increases, consumers look for **alternative eco-friendly products**, especially the new generations which are values-oriented shoppers, motivated by renewable ingredients, plant-based foods, and sustainable production methods when make purchasing decisions (Purcell, 2020). Global consumers who expect companies to invest in sustainability increased from 65% in 2018 to 87% in 2019. About half of consumers are willing to pay extra for products devoted to solving sustainability issues. But while appeal for second-hand products is on the rise, convenience and affordability still play a role in consumers' buying decisions (Angus & Westbrook, 2020). Price is one of the most important considerations of fresh food purchases, with 92% of consumers citing cost as an important aspect in their purchasing decisions (Renner et al., 2019).

Considering sustainability and efficient use of resources, food industry is emphasising on the recovery, recycling and upgrading of food wastes, which can be promising sources of valuable compounds, considering their favourable technological, nutritional and functional properties (Otles et al., 2015). Sustainable products can be defined as products with positive, social and/or environmental attributes, with the latter receiving more attention in the literature (92%), followed by economic (54%) and social (51%) sustainability dimensions (Kamble et al., 2020).

Sustainability is a growing concern in the food industry, partly because it plays a significant role in environmental impact, being responsible for around 30% of total greenhouse gas emissions. To help tackle this challenge, companies are implementing green innovations aiming to secure several advantages, including the ability to obtain a higher price for their eco-friendly products, an improvement in their corporate image, the development of new markets, and a competitive edge. In fact, around half of the consumers are willing to pay more for sustainable products (Schaufele & Hamm, 2017).

Food processing constitutes 25% of all water consumption worldwide and 50 - 80% of all water used in industrial countries. Pursuing more sustainable practices, the food industry responds by developing strategies and adopting procedures to minimise the impact of food processing into the environment. The use of **eco-friendly technologies** for the extraction of functional components could be valuable tools to enhance extraction yields of valuable compounds from materials, contributing to the full valorisation of resources, thus promoting sustainable food industry (Putnik et al., 2018).

These technologies (e.g. microwave hydrodiffusion and gravity - MHG, ultrasound-assisted extraction - UAE) are time and energy saving procedures, increasing the production efficiency and reducing water and solvents need, simplifying manipulation and processing costs (Turrini et al., 2019). These techniques are easy to handle, require moderate solvent (Galanakis, 2012) and have recently

been applied for the extraction of polyphenols from grape berries, potato by-products and seaweed (Al Bittar et al., 2013; Paleologou et al., 2016; López-Hortas et al., 2018).

Among these green extraction technologies, subcritical water extraction (SWE) which uses hot water under pressure, has recently emerged as a useful and environmentally friendly tool due to its food compatibility, low-cost, and possible combination with other technologies (Flórez-Fernández et al., 2017). Subcritical water extraction has been widely used to extract different compounds from several matrices such as peanut shells, potato peel and seaweed (Rico et al., 2018; Torres et al., 2020; Flórez-Fernández et al., 2019).

1.1.2.1. By-products of the food industries

Resources scarcity have led consumers, researchers and companies to search for viable alternatives. Food waste is a significant global problem, with estimates suggesting that one third of edible food produced for human consumption is wasted globally each year (FAO, 2011). Food industries generate high amounts of food **by-products** (e.g. rice bran, potato peel, apple pomace, cheese whey) which are promising sources of valuable compounds such as dietary fibre, antioxidants, fatty acids, antimicrobials and minerals. These materials are excellent natural sources with high potential for use in different applications as innovative food ingredients, considering their favourable technological, nutritional and functional properties (Galanakis, 2012; Lai et al., 2017).

Following the European Commission strategy on **Circular Economy**, transforming wastes into high value-added products, and increasing globalised market for secondary raw materials (European Commission, 2015a), the development of added-value products based on food by-products is currently an important research topic to improve the sustainability of the food chain. Many researchers have investigated the potential use of industrial by-products (e.g. fish skin, apple and tomato pomace, grape seeds, coffee husk) for food applications (O'Shea et al., 2012; Pal & Suresh, 2016; Iriondo-DeHond et al., 2019).

Annually, European countries are responsible for the production of 4.5 million ton of paddy rice, which leads to high quantities of rice by-products (up to 40%), including broken rice, and rice bran, mainly forwarded to feed. These materials with high nutritional value, rich in fibres, minerals and antioxidants can still be of value to human food, making the product life cycle more efficient. Several studies have been published on the development of sweet cookies, extruded snacks and noodles using broken rice flour (Tavares et al., 2016; Oliveira et al., 2019; Ahmed et al. 2015).

Potato peel valorisation falls within the food waste group. Potato is most used as a source for industrial starch production, since the extraction procedure is simpler in comparison to cereal starches (Torres et al., 2018; Torres et al., 2020). Up to 30% discarding degree due to undersized potatoes, which currently have a low added value being used primarily for animal feed (Priedniece et al., 2017).

Also, potatoes peels are rich sources of phenolic compounds and oligosaccharides and their biological properties have been extensively studied (Akyol et al., 2016; Riciputi et al., 2018; Jeddou et al., 2018).

1.1.2.2. Alternative sources of ingredients

Driven by the growing global protein demand, increasing concerns over the environmental effects of livestock farming and for animal welfare, and the rise of *clean eating,* consumers are adopting plant-based diets (Caro et al., 2014; Asioli et al., 2017; Reipurth et al., 2019). Whether it comprises a vegan diet, eating as many fruits and vegetables as possible, or consuming minimally processed foods that come from plants, avoiding animal meat is consensual among consumers. This trend (Lee, 2019; Purcell, 2020) has led researchers and food processors to explore **alternative plant-based protein** sources, namely oat, lupin, potato, peas and algae (Pintado et al., 2016; Schmidt et al., 2018; García-Segovia et al., 2020; Niccolai et al., 2019). Among them, potato protein is found to be more favourably accepted by consumers compared to the other plant-based protein ingredients, due to familiarity issues (Aschemann-Witzel & Peschel, 2019). Also, the food and technology industries are working together to create new plant-based substitutes to cheese, meat and eggs (e.g. Tyson Foods and Nestlé) (EIU, 2018) and novel protein sources like insects, algae, duckweed, and rapeseed are expected to enter the European feed and food market (Rumpold & Schlüter, 2013; van der Spiegel et al., 2013). Protein-packed noodles also are on the rise, and more noodles made from seafood, or containing minerals and dietary fibre are anticipated to reach the market (Purcell, 2020).

Microalgae are a biological resource with a huge potential as a food ingredient, representing one of the most promising sources for new food products and applications, enhancing their nutritional and technological properties, due to their well-balanced chemical composition rich in proteins, essential amino acids, polysaccharides, glucans, fibres, polyunsaturated fatty acids. Moreover, microalgae are extremely rich in natural pigments (chlorophyll, phycobiliproteins, carotenoids) widely recognized as having health-beneficent effects namely protection from oxidative stress acting as antioxidants (Becker, 2004; Plaza et al., 2009; Hongsthong & Bunnag, 2009).

In Europe, the use of microalgae as food ingredients is legislated by the Regulation (UE) 2015/2283, and currently only *Odontella aurita* and *Tetraselmis chuii* have been approved as novel foods, and the oils from *Schizochytrium* or *Ulkenia* and astaxanthin from *Haematococcus pluvialis* approved as novel food ingredients (European Commission, 2015b; European Commission, 2017; García et al., 2017). However, foods commercialised in at least one Member State before the Regulation on Novel Foods on 15 May 1997, are on the EU market under the *"principle of mutual recognition"*. This is the case of *Chlorella vulgaris* and *Arthrospira platensis* (commonly known as Spirulina), which has high protein content (Tibbetts et al., 2015; Batista et al., 2017), and acts as a pigment agent due to its phycocyanin contents.

Although there are still several challenges that need to be overcome in terms of low production capacities, high production costs, and intense colour and a fishy taste and aroma, several food products have been already launched in the market (e.g. instant soup, cereal bars, smoothies) (Lafarga, 2019), in line with the current trends on sustainability, convenience and food functionality.

In the last years, due to sustainability concerns and driven by consumers demands on new functional products, also seaweed have received special attention as alternative, renewable and valuable resources. **Macroalgae** (or seaweeds) are a very heterogeneous group of aquatic photosynthetic organisms classified according to the presence of specific pigments in red (Rhodophyta), green (Chlorophyta) and brown (Phaeophyta). Brown seaweeds are the major class contributing close to 59% of the total culture production in 2006 followed by red (40%) and green (< 1%) seaweeds (D'Orazio et al., 2012).

Although macroalgae have been traditionally used for food and medicine in Asian countries, in Europe they have been only valued through its extracts, which include hydrocolloids (agar, carrageen and alginate) with technological advantages when used as ingredients (gelling, thickening and emulsifying agents) in food and beverage production (Kumar et al., 2008; Cofrades, 2013; Pereira, 2016). More recently, macroalgae have gained much attention by researchers and consumers due to sustainability trends and health concerns (Sudhakar et al., 2018). From a nutritional point of view, seaweeds are recognised as an important source of minerals, vitamins, proteins and indigestible carbohydrates, and have low lipidic content (Kumar et al., 2008). Several studies reported that these organisms present antioxidant, antimicrobial and antitumoral properties, which could provide new compounds for food, pharmaceutical and cosmetic industries (Fernandes et al., 2016; Flórez et al., 2017).

Nowadays, the European seaweed processing industry is divided into two main categories: those producing alginic acid and those producing products for agriculture (fertilizer, animal feed). Only a few seaweeds are destined for human consumption (e.g. *Laminaria ochroleuca*, *Undaria pinnatifida*, *Palmaria palmata*, *Saccharina latissima*), being harvested manually and dried in an artisanal way. The main constraints on the development of seaweed farming have been the lack of markets and the high cost of European production compared to Asian production (NETALGAE, 2012).

Globally, in the last years an interesting and important trend in terms of the sustainability (both ecological and economic) of the seaweed industry is the growth in seaweed farming as well as a decline in seaweed wild harvest: from 52 % in 1995 to 4% in 2012. Much of this change is being driven by the growth in the seaweed farming industries of *Laminaria*, *Undaria*, *Gracilaria*, and *Porphyra* in China and *Eucheuma*/ *Kappaphycus* in Indonesia (White & Wilson, 2015).

In this sense, the utilization of seaweeds and microalgae as sustainable food sources would also require promoting them for human consumption at a global level through innovation and identification of niche markets.

1.1.3. Healthy Foods

Innovative food products with health-promoting components have gained growing interest within the research community, food industry, and consumers (Khan et al., 2013; Küster-Boluda & Vidal-Capilla, 2017; Santeramo et al., 2018; Hung & Verbeke, 2019). The increasing awareness of the relationship between nutrition and health led consumers to be more concerned with their food choices, and functional foods have taken a more prominent role in the diet.

Functional food is any fresh or processed food that is claimed to have a health-promoting and/or disease-preventing property beyond the basic nutritional function of supplying nutrients. These foods may help prevent disease, reduce the risk of developing disease, or enhance health (Arias-Aranda & Romerosa-Martínez, 2010). To differentiate these functional foods from the ingredients that improve food technological performance, there is a tendency to apply this concept with the designation of healthy foods.

Currently, consumers want healthy products that also help reducing disease risk, without failing to be incredibly delicious and appealing. The healthy food market is continuously growing, being expected an increase of 8 % annually in functional food sales in Europe, between 2019 and 2023 (Statista, 2019). A recent study with the Norwegian population, Nystrand & Olsen (2020) found that consumers' attitudes towards eating healthy foods are driven by utilitarian motivations as opposed to hedonic eating values, suggesting that the food industry could benefit from improving hedonic attributes of healthy foods for targeting new consumer segments.

Within the healthy food market, the dietary fibre enrichment of foods was one of the first and the most common, and this trend is expected to continue growing at a 12% year rate until 2024 (Statista, 2018). The health benefits attributed to the ingestion of dietary fibre are well established, including the reduction of the glycemic index of foods which diabetes, to the prevention of constipation, reduced risk for obesity, hypertension and atherosclerosis (Elleuch et al., 2011; Bustos et al., 2013; Soliman, 2019). In general, food with high dietary fibre content include cereal products, fruits and vegetables. In 2016, 38% of the consumers look for *high fibre* in product's label (Ringquist et al., 2016), with new research continually emerging to reinforce this trend (Lee, 2019). This renewed interest in **fibre**, going beyond digestive health benefits, is expected to drive fibre claims and new fibre applications (Lee, 2019). Aligning this trend with sustainability issues, the food industry is looking for low cost sources of dietary fibre, such as peels and pomace of a wide range of fruits and vegetables (e.g. orange, carrot, blueberry, potato) (Xie et al., 2017; Garcia-Amezquita et al., 2018; Curutchet et al., 2019). This approach could be an opportunity for industries to reduce wastes and generating added-value products.

Psyllium husk is another fibre source that gained attention from the research community especially since its health claim from FDA (FDA, 2012). This recognised functional ingredient is associated with the reduction of coronary heart disease risk, and has also documented its

hypocholesterolemic effect and improvement of glycaemic control (Singh, 2007; Abutair et al., 2016), Due to its high water absorption (Raymundo et al., 2014) and gelling properties it has high potential to be a structure builder that mimics the gluten matrix, as already been assessed for GF bread dough (Cappa et al., 2013).

1.2. Objectives

The main objectives of this thesis are related to three main vectors:

1. Concept of circular economy, sustainability and efficiency of the resources, adding value to food industries by-products, namely broken rice and potato peel, and the exploitation of new sources of ingredients like microalgae and seaweed.
2. Complexity of producing gluten-free (GF) fresh pasta, as gluten is the major matrix to support structure of pasta material
3. Nutritional balance of the GF products by innovative ingredients with bioactive potential (*Arthrospira platensis*, *Laminaria ochroleuca* and potato peel) to obtain sensory attractive GF products

Other subsequent objectives are:

- Understanding the thermal processing of rice flour: pre-gelatinisation phenomena and its influence in the formation of structures
- Study the impact of rice variety on thermal processing of rice flour
- Optimize the GF pasta processing conditions to obtain products similar to its gluten counterparts
- Determine the thermorheological behaviour of *Psyllium* husk, to define its optimal processing conditions to build-up structure of GF pasta
- Study the incorporation of sustainable ingredients (*Arthrospira platensis, Laminaria ochroleuca,* potato peel) to improve the nutritional performance of the GF pasta products
- Evaluate the *in vitro* digestibility of some developed pastas

1.3. References

Abutair, A.S., Naser, I.A., Hamed, A.T. (2016). Soluble fibers from psyllium improve glycemic response and body weight among diabetes type 2 patients (randomized control trial). *Nutrition Journal*, 15, 86. https://doi.org/10.1186/s12937-016-0207-4

Ahmed, J., Al-Jassar, S. & Thomas, L. (2015). A comparison in rheological, thermal, and structural properties between Indian Basmati and Egyptian Giza rice flour dispersions as influenced by particle size. *Food Hydrocolloids*, 48, 72–83. https://doi.org/10.1016/j.foodhyd.2015.02.012

Akyol, H., Riciputi, Y., Capanoglu, E., Caboni, M. F., Verardo, V. (2016). Phenolic compounds in the potato and its byproducts: An overview. *International Journal of Molecular Sciences*, 17(6), 835. https://doi.org/10.3390/ijms17060835

Al Bittar, S., Périno-Issartier, S., Dangles, O., Chemat, F. (2013). An innovative grape juice enriched in polyphenols by microwave-assisted extraction. *Food Chemistry*, 141, 3268–3272. https://doi.org/10.1016/j.foodchem.2013.05.134

Alexandratos, N., Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision. Agricultural Development Economics Division (ESA), Paper No. 12–03, Food and Agriculture Organization, United Nations, Rome

Allen, B., Orfila, C. (2018). The Availability and Nutritional Adequacy of Gluten-Free Bread and Pasta. *Nutrients*, 10, 1370. https://doi.org/10.3390/nu10101370

Amos, C., Pentina, I., Hawkins, T. G., Davis, N. (2014). "Natural" labeling and consumers' sentimental pastoral notion. *The Journal of Product and Brand Management*, 23(4/5), 268–281. https://doi.org/10.1108/JPBM-03-2014-0516

Angus, A., Westbrook, G. (2020). Top 10 Global Consumer Trends 2020. Euromonitor International. URL: https://go.euromonitor.com/white-paper-EC-2020-Top-10-Global-Consumer-Trends.html?utm_source=press_release&utm_medium=PR&utm_campaign=CT_WP_20_01_14_Top_10_GCT_2020

Arias-Aranda, D., Romerosa-Martínez, M.M. (2010). Innovation in the functional foods industry in a peripheral region of the European Union: Andalusia (Spain). *Food Policy*, 35, 240–246. https://doi.org/10.1016/j.foodpol.2009.12.008

Aschemann-Witzel, J., Peschel, A.O. (2019). Consumer perception of plant-based proteins: The value of source transparency for alternative protein ingredients. *Food Hydrocolloids*, 96, 20-28. https://doi.org/10.1016/j.foodhyd.2019.05.006

Aschemann-Witzel, J., Varela, P., Peschel, A.O. (2019). Consumers' categorization of food ingredients: Do consumers perceive them as 'clean label' producers expect? An exploration with projective mapping. *Food Quality and Preference*, 71, 117–128. https://doi.org/10.1016/j.foodqual.2018.06.003

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., Varela, P. (2017). Making sense of the ''clean label'' trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International*, 99, 58–71. https://doi.org/10.1016/j.foodres.2017.07.022

Barreiro-Hurlé, J., Gracia, A., de-Magistris, T. (2010). Does nutrition information on food products lead to healthier food choices? *Food Policy*, 35, 221–229. https://doi.org/10.1016/j.foodpol.2009.12.006

Batista, A.P., Niccolai, A., Fradinho, P., Fragoso, S., Bursic, I., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A. (2017). Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. *Algal Research*, 26, 161-171. https://doi.org/10.1016/j.algal.2017.07.017

Becker, W. (2004). Microalgae in human and animal nutrition. In: A. Richmond (Ed.), *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Blackwell Publishing Ltd., Oxford, pp. 312–351.

Batt, P.J. (2018). Responding to the challenges presented by global megatrends. *Acta Horticulture*, 1205, 1-12. https://doi.org/10.17660/ActaHortic.2018.1205.1

Bollani, L., Bonadonna, A., Peira, G. (2019). The Millennials' Concept of Sustainability in the Food Sector. *Sustainability*, 11, 2984. https://doi.org/10.3390/su11102984

Bowman, S.A. (2006). A comparison of the socioeconomic characteristics, dietary practices, and health status of women food shoppers with different food price attitudes. *Nutrition Research*, 26, 318–324. https://doi.org/10.1016/j.nutres.2006.06.012

Bustamante, M.A., Fernández-Gil, M.P., Churruca, I., Miranda, J., Lasa, A., Navarro, V., Simón, E. (2017). Evolution of Gluten Content in Cereal-Based Gluten-Free Products: An Overview from 1998 to 2016. *Nutrients,* 9, 21. https://doi.org/10.3390/nu9010021

Bustos, M.C., Perez, G.T., León, A.E. (2013). Combination of resistant starches types II and IV with minimal amounts of oat bran yields good quality, low glycaemic index pasta. *International Journal of Food Science and Technology*, 48, 309–315. https://doi.org/10.1111/j.1365-2621.2012.03188.x

Cappa, C., Lucisano, M., Mariotti, M. (2013). Influence of Psyllium, sugar beet fibre and water on gluten-free dough properties and bread quality. *Carbohydrate Polymers*, 98, 1657– 1666. https://doi.org/10.1016/j.carbpol.2013.08.007

Caro, D., Davis, S. Bastianoni, S. (2014). Global and Regional Trends in Greenhouse Gas Emissions from Livestock. *Climatic Change*, 126, 203-216. https://doi.org/10.1007/s10584-014-1197-x

Cecchini, M., Warin, L. (2016). Impact of Food Labelling Systems on Food Choices and Eating Behaviors: A Systematic Review and Meta-Analysis of Randomized Studies: Impact of Food Labelling. *Obesity Reviews*, 17, 201–210. https://doi.org/10.1111/obr.12364

Chauke, D.X., Duh, H.I. (2019). Marketing and Socio-psychological Factors Influencing Organic Food Purchase and Post-Purchase Outcomes, *Journal of Food Products Marketing*, 25(9), 896-920. https://doi.org/10.1080/10454446.2019.1697980

Cofrades, S., Serdaroğlu, M., Jiménez-Colmenero, F. (2013). Design of Healthier Foods and Beverages Containing Whole Algae. In: H. Domínguez (Ed.), *Functional Ingredients from Algae for Foods and Nutraceuticals*, Woodhead Publishing, Cambridge, pp. 609–633.

Curutchet, A., Cozzano, S., Tárrega, A., Arcia, P. (2019). Blueberry pomace as a source of antioxidant fibre in cookies: Consumer's expectations and critical attributes for developing a new product. *Food*

Science and Technology International, 25(8), 642–648. https://doi.org/10.1177/1082013219853489

D'Orazio, N., Gemello, E., Gammone, M.A., de Girolamo, M., Ficoneri, C., Riccioni, G. (2012). Fucoxantin: A Treasure from the Sea. *Marine Drugs*, 10, 604-616. https://doi.org/10.3390/md10030604

EIU (2018). Global Food Trends to 2030: with a closer look at the GCC. The Economist Intelligence Unit. Dubai Industrial Park report. URL: http://dubaiindustrialcity.ae/reports/Global-Food-Trends-To-2030.php?csrt=15676586929829302087

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry,* 124, 411–421. https://doi.org/10.1016/j.foodchem.2010.06.077

EUFIC (2020). Food Choice - Why do we eat what we eat? European Food Information Council web site. URL: https://www.eufic.org/en/healthy-living/category/food-choice/

European Commission (2015a). Closing the loop – an EU action plan for the circular economy. Brussels, COM(2015)614 final. URL: https://eur-lex.europa.eu/resource.html?uri=cellar:8a8ef5e8-99a0-11e5-b3b7-01aa75ed71a1.0012.02/DOC_1&format=PDF

European Commission (2015b). Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods. Official Journal of the European Communities. URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2283&qid=1551104546958&from=PT

European Commission (2017). Regulation (EU) 2017/2470 of the European Parliament and of the Council of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. Official Journal of the European Communities. URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R228 3&qid=1551104546958&from=PT

FAO (2011). Global food losses and food waste – Extent, causes and prevention. Food and Agriculture Organization of the United Nations. Rome. URL: http://www.fao.org/3/mb060e/mb060e.pdf

FDA (2012). CFR – code of federal regulations title 21. U.S. Food and Drug Administration web site. URL: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr= 101.81

Fernandes, F., Barbosa, M., Oliveira, A.P., Azevedo, I.C., Sousa-Pinto, I., Valentão, P., Andrade, P.B. (2016). The pigments of kelps (*Ochrophyta*) as part of the flexible response to highly variable marine environments. *Journal of Applied Phycology*, 28, 3689–3696. https://doi.org/10.1007/s10811-016-0883-7

Flórez, N., Gonzalez-Muñoz, M.J., Ribeiro, D., Fernandes, E., Dominguez, H., Freitas, M. (2017) Algae polysaccharides' chemical characterization and their role in the inflammatory process. *Current Medicinal Chemistry*, 24, 149–175. https://doi.org/10.2174/0929867323666161028160416

Flórez-Fernández, N., González-Muñoz, M.J., Domínguez, H. (2017). Feasibility of posthydrolysis processing of hydrothermal extracts from *Sargassum muticum*. *Algal Research*, 27, 73–81. https://doi.org/10.1016/j.algal.2017.08.002

Flórez-Fernández, N., Torres, M.D., González-Muñoz, M.J., Domínguez, H. (2019). Recovery of bioactive and gelling extracts from edible brown seaweed *Laminaria ochroleuca* by non-isothermal autohydrolysis. *Food Chemistry*, 277, 353-361. https://doi.org/10.1016/j.foodchem.2018.10.096

Food Ingredients First (2019). Natural Flavors Innovation. URL: https://www.foodingredientsfirst.com/analysis-feature/natural_flavors_dec_2019.html

Galanakis, C.M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, 26, 68-87. https://doi.org/10.1016/j.tifs.2012.03.003

García, J.L., de Vicente, M., Galán, B. (2017). Microalgae, old sustainable food and fashion nutraceuticals. *Microbial Biotechnology*, 10(5), 1017–1024. https://doi.org/10.1111/1751-7915.12800

Garcia-Amezquita, L.E., Tejada-Ortigoza, V., Serna-Saldivar, S.O., Welti-Chanes, J. (2018). Dietary Fiber Concentrates from Fruit and Vegetable By-products: Processing, Modification, and Application as Functional Ingredients. *Food and Bioprocess Technology,* 11, 1439–1463. https://doi.org/10.1007/s11947-018-2117-2

García-Segovia, P., Igual, M., Noguerol, A.T., Martínez-Monzó, J. (2020). Use of insects and pea powder as alternative protein and mineral sources in extruded snacks. *European Food Research and Technology*. https://doi.org/10.1007/s00217-020-03441-y

Gorski Findling, M.T., Werth, P.M., Musicus, A.A., Bragg, M.A., Grahame, D.J., Elbel, B., Roberto, C.A. (2018). Comparing five front-of-pack nutrition labels' influence on consumers' perceptions and purchase intentions. *Preventive Medicine,* 106, 114–121. https://doi.org/10.1016/j.ypmed.2017.10.022

Gracia, A., de Magistris, T. (2016). Consumer preferences for food labeling: What ranks first? *Food Control*, 61, 39–46. https://doi.org/10.1016/j.foodcont.2015.09.023

Hendry, N. (2019). Innovation Opportunities Across the Global Food Landscape. Global Data Report. Portugal Foods.

Hongsthong, A., Bunnag, B. (2009). Biochemistry, biotechnology and applications. In: P.M. Gault, H.J. Marler (Eds.), *Handbook on Cyanobacteria*, Nova Science Publishers, New York, pp. 51–103.

Hung, Y., Verbeke, W. (2019). Consumer evaluation, use and health relevance of health claims in the European Union. *Food Quality and Preference*, 74, 88–99. https://doi.org/10.1016/j.foodqual.2019.01.002

Iriondo-DeHond, A., García, N.A., Fernandez-Gomez, B., Guisantes-Batan, E., Escobar, F.V., Blanch, G.P., San Andres, M.I., Sanchez-Fortun, S., del Castillo, M.D. (2019). Validation of coffee by-products as novel food ingredients. *Innovative Food Science and Emerging Technologies*, 51, 194–204. https://doi.org/10.1016/j.ifset.2018.06.010

Jeddou, K.B., Bouaziz, F., Helbert, C.B., Nouri-Ellouz, O., Maktouf, S., Ellouz-Chaabouni, S., Ellouz-Ghorbel, R. (2018). Structural, functional, and biological properties of potato peel oligosaccharides. *International Journal of Biological Macromolecules,* 112, 1146–1155. https://doi.org/10.1016/j.ijbiomac.2018.02.004

Kamble, S.S., Gunasekaran, A., Gawankar, S.A. (2020). Achieving sustainable performance in a data-driven agriculture supply chain: A review for research and applications. *International Journal of Production Economics,* 219, 179–194. https://doi.org/10.1016/j.ijpe.2019.05.022

Khan, R., Grigor, J., Winger, R., Win, A. (2013). Functional food product development-opportunities and challenges for food manufacturers. *Trends in Food Science & Technology*, 30, 27–37. https://doi.org/10.1016/j.tifs.2012.11.004

Kinsey, L., Burden, S.T., Bannerman, E. (2008). A dietary survey to determine if patients with coeliac disease are meeting current healthy eating guidelines and how their diet compares to that of the British general population. *European Journal of Clinical Nutrition*, 62, 1333–1342. https://doi.org/10.1038/sj.ejcn.1602856

Kumar, S.C., Ganesan, P., Suresh, P.V., Bhaskar, N. (2008). Seaweeds as a source of nutritionally beneficial compounds – A review. *Journal of Food Science and Technology,* 45(1), 1–13. https://scinapse.io/papers/90390535

Küster-Boluda, I., Vidal-Capilla, I. (2017). Consumer attitudes in the election of functional foods. *Spanish Journal of Marketing-ESIC*, 21(S1), 65–79. https://doi.org/10.1016/j.sjme.2017.05.002

Lafarga, T. (2019). Effect of microalgal biomass incorporation into foods: Nutritional and sensorial attributes of the end products. Algal Research, 41, 101566. https://doi.org/10.1016/j.algal.2019.101566

Lai, W.T., Khong, N.M.H., Lim, S.S., Hee, Y.Y., Sim, B.I., Lau, K.Y., Lai, O.M. (2017). A review: Modified agricultural by-products for the development and fortification of food products and nutraceuticals. Trends in Food Science & Technology, 59, 148-160. https://doi.org/10.1016/j.tifs.2016.11.014

Lee, V. (2019) Top Ten Trends for 2019. Innova Market Insights. URL: https://euagenda.eu/publications/top-ten-food-trends-for-2019

López-Hortas, L., Gannon, L., Moreira, R., Chenlo, F., Domínguez, H., Torres, M.D. (2018). Microwave hydrodiffusion and gravity (MHG) processing of *Laminaria ochroleuca* brown seaweed. *Journal of Cleaner Production*, 197, 1108-1116. https://doi.org/10.1016/j.jclepro.2018.06.274

Mintel (2018). Mintel's The Natural/Organic Shopper Canada 2018 report. URL: https://www.mintel.com/press-centre/food-and-drink/natural-fooddrink-launches-with-gmo-free-claims-grew-366-in-canada-in-the-last-10-years

Missbach, B., Schwingshackl, L., Billmann, A., Mystek, A., Hickelsberger, M., Bauer, G., König, J. (2015). Gluten-free food database: the nutritional quality and cost of packaged gluten-free foods. *PeerJ*, 3, e1337. https://doi.org/10.7717/peerj.1337

Mordor Intelligence (2020). Free-From Food Market – Growth, Trends, and Forecast (2020-2025). URL: https://www.mordorintelligence.com/industry-reports/free-from-food-market

NETALGAE (2012). *Seaweed Industry in Europe*. URL: http://www.seaweed.ie/irish_seaweed_contacts/doc/Filieres_12p_UK.pdf

Ni Mhurchu, C., Eyles, H., Jiang, Y., Blakely, T. (2018). Do nutrition labels influence healthier food choices? Analysis of label viewing behaviour and subsequent food purchases in a labelling intervention trial. *Appetite*, 121, 360-365. https://doi.org/10.1016/j.appet.2017.11.105

Niccolai, A., Chini Zittelli, G., Rodolfi, L., Biondi, N., Tredici, M.R. (2019). Microalgae of interest as food source: Biochemical composition and digestibility. *Algal Research,* 42, 101617. https://doi.org/10.1016/j.algal.2019.101617

NRA (2018). The State of Restaurant Sustainability 2018 edition. National Restaurant Association. URL: https://www.restaurant.org/Downloads/PDFs/sustainability/restaurant_sustainability_research_report_2018.pdf

NRF (2020). Consumer View Winter 2020: Convenience and the Consumer. National Retail Federation web site. URL: https://nrf.com/research/consumer-view-winter-2020

Nystrand, B.T., Olsen, S.O. (2020). Consumers' attitudes and intentions toward consuming functional foods in Norway. *Food Quality and Preference,* 80, 103827. https://doi.org/10.1016/j.foodqual.2019.103827

O'Shea, N., Arendt, E.K., Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technologies,* 16, 1–10. https://doi.org/10.1016/j.ifset.2012.06.002

Oliveira, A.R., Ribeiro, A.E.C., Oliveira, E.R., Mendes da Silva, A.C., Soares Júnior, M., Caliari, M. (2019). Broken rice grains pregelatinized flours incorporated with lyophilized açaí pulp and the effect of extrusion on their physicochemical properties. *Journal of Food Science and Technology*, 56(3), 1337–1348. https://doi.org/10.1007/s13197-019-03606-y

Otles, S., Despoudi, S., Bucatariu, C., Kartal, C. (2015). Food waste management, valorization, and sustainability in the food industry. In: C.M. Galanakis (Ed.), *Food Waste Recovery: Processing Technologies and Industrial Techniques*, Academic Press, London, pp. 3–23. https://doi.org/10.1016/b978-0-12-800351-0.00001-8

Pal, K.G., Suresh, P.V. (2016). Sustainable valorisation of seafood by-products: Recovery of collagen and development of collagen-based novel functional food ingredients. *Innovative Food Science and Emerging Technologies*, 37, 201–215. https://doi.org/10.1016/j.ifset.2016.03.015

Pala, V., Sieri, S., Masala, G., Palli, D., Panico, S., Vineis, P., Sacerdote, C., Mattiello, A., Galasso, R., Salvini, S., Ceroti, M., Berrino, F., Fusconi, E., Tumino, R., Frasca, G., Riboli, E., Trichopoulou, A., Baibas, N., Krogh, V. (2006). Associations between dietary pattern and lifestyle, anthropometry and other health indicators in the elderly participants of the EPIC-Italy cohort. *Nutrition, Metabolism & Cardiovascular Diseases*, 16(3), 186-201. https://doi.org/10.1016/j.numecd.2005.05.009

Paleologou, I., Vasiliou, A., Grigorakis, S., Makris, D.P. (2016). Optimisation of a green ultrasound-assisted extraction process for potato peel (*Solanum tuberosum*) polyphenols using bio-solvents and response surface methodology. *Biomass Conversion and Biorefinery*, 6, 289–299. https://doi.org/10.1007/s13399-015-0181-7

Pereira, L. (2016). *Edible Seaweed of the World*. CRC Press: Boca Raton, USA.

Pintado, T., Herrero, A.M., Jiménez-Colmenero, F., Ruiz-Capillas, C. (2016). Emulsion gels as potential fat replacers delivering β-glucan and healthy lipid content for food applications. *Journal of Food Science & Technology*, 53(12), 4336–4347. https://doi.org/10.1007/s13197-016-2432-4

Plaza, M., Herrero, M., Cifuentes, A., Ibanez, E. (2009). Innovative natural functional ingredients from microalgae, *Journal of Agricultural and Food Chemistry*, 57, 7159–7170. https://doi.org/10.1021/jf901070g

Priedniece, V., Spalvins, K., Ivanovs, K., Pubule, J., Blumberga, D. (2017). Bioproducts from potatoes. A review. *Environmental and Climate Technologies*, 21, 18–27. https://doi.org/10.1515/rtuect-2017-0013

Purcell, D. (2020). Trend Report: Top Food Trends for 2020. Specialty Food Magazine. URL: https://www.specialtyfood.com/news/article/trend-report-top-food-trends-2020

Putnik, P., Lorenzo, J.M., Barba, F.J., Roohinejad, S., Jambrak, A.R., Granato, D., Montesano, D., Kovačević, D.B. (2018). *Foods*, 7, 106, https://doi.org/10.3390/foods7070106

Ramankutty, N., Mehrabi, Z., Waha, K., Jarvis, L., Kremen, C., Herrero, M., Rieseberg, L.H. (2018). Trends in Global Agricultural Land Use: Implications for Environmental Health and Food Security. *Annual Review of Plant Biology,* 69, 14.1–14.27. https://doi.org/10.1146/annurev-arplant-042817-040256

Raymundo, A., Fradinho, P., Nunes, M.C. (2014). Effect of Psyllium fibre content on the textural and rheological characteristics of biscuit and biscuit dough. *Bioactive Carbohydrates and Dietary Fibre*, 3(2), 96-105. https://doi.org/10.1016/j.bcdf.2014.03.001

Reipurth, M.F.S., Hørby, L., Gregersen, C.G., Bonke, A., Perez Cueto, F.J.A. (2019). Barriers and facilitators towards adopting a more plant-based diet in a sample of Danish consumers. *Food Quality and Preference*, 73, 288-292. https://doi.org/10.1016/j.foodqual.2018.10

Renner, B., Baker, B., Fedder, C., Joshi, S., Upadhyaya, J. (2019). The future of fresh: strategies to realize value in the fresh food category. Deloitte Development LLC. URL: https://www2.deloitte.com/us/en/insights/industry/retail-distribution/future-of-fresh-food-sales-consumer-demand-strategi es.html

Riciputi, Y., Diaz-de-Cerio, E., Akyol, H., Capanoglu, E., Cerretani, L., Caboni, M.F., Verardo, V. (2018). Establishment of ultrasound-assisted extraction of phenolic compounds from industrial potato by-products using response surface methodology. *Food Chemistry,* 269, 258–263. https://doi.org/10.1016/j.foodchem.2018.06.154

Rico, X., Gullón, B., Alonso, J.L., Parajó, J.C., Yáñez, R. (2018). Valorization of peanut shells: Manufacture of bioactive oligosaccharides. *Carbohydrate Polymers*, 183, 21-28. https://doi.org/10.1016/j.carbpol.2017.11.009

Ringquist, J., Phillips, T., Renner, B., Sides, R., Stuart, K., Baum, M., Flannery, J. (2016). Capitalizing on the shifting consumer food value equation. Deloitte Development LLC. URL: https://www2.deloitte.com/content/dam/Deloitte/us/Documents/consumer-business/us-fmi-gma-report.pdf

Rumpold, B.A., Schlüter, O.K. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies,* 17, 1–11. https://doi.org/10.1016/j.ifset.2012.11.005

Santeramo, F.G., Carluccib, D., De Devitiisa, B., Secciaa, A., Stasia, A., Viscecchiaa, R., Nardone, G. (2018). Emerging trends in European food, diets and food industry. *Food Research International*, 104, 39–47. https://doi.org/10.1016/j.foodres.2017.10.039

Schaufele, I., Hamm, U. (2017). Consumers' perceptions, preferences and willingness to-pay for wine with sustainability characteristics: a review. *Journal of Cleaner Production*, 147, 379-394. https://doi.org/10.1016/j.jclepro.2017.01.118

Schmidt, J.M., Damgaard, H., Greve-Poulsen, M., Larsen, L.B., Hammershøj, M. (2018). Foam and emulsion properties of potato protein isolate and purified fractions. *Food Hydrocolloids*, 74, 367-378. https://doi.org/10.1016/j.foodhyd.2017.07.032

Seifer, D. (2019). 2020 Predictions: consumer insights. *Prepared Foods,* 188, I.12. URL: https://www.preparedfoods.com/articles/123302-four-key-consumer-behaviors-driving-purchase-patterns-preferences

Singh, B. (2007). Psyllium as therapeutic and drug delivery agent. *International Journal of Pharmaceutics*, 334: 1–14. https://doi.org/10.1016/j.ijpharm.2007.01.028

Singh, J., Whelan, K. (2011). Limited availability and higher cost of gluten-free foods. *Journal of Human Nutrition and Dietetics*, 24, 479–486. https://doi.org/10.1111/j.1365-277X.2011.01160.x

Soliman, G.A. (2019). Dietary Fiber, Atherosclerosis, and Cardiovascular Disease. *Nutrients*, 11, 1155. https://doi.org/10.3390/nu11051155

Statista (2018). Gluten-free per capita spending forecast in leading markets in Europe in 2015 and 2020. URL: https://www.statista.com/statistics/646341/gluten-free-per-capita-spending-european-union-eu/

Statista (2019). Sales of functional food ingredients worldwide from 2016 to 2023, by region. URL: https://www.statista.com/statistics/253083/global-functional-food-sales-by-country/

Sudhakar, K., Mamat, R., Samykano, M., Azmi, W.H., Ishak, W.F.W., Yusaf, T. (2018). An overview of marine macroalgae as bioresource. *Renewable and Sustainable Energy Reviews,* 91, 165-179. https://doi.org/10.1016/j.rser.2018.03.100

Tavares, B.O., Silva, E.P., Silva, V.S.N., Soares Júnior, M.S., Ida, E.I., Damiani, C. (2016). Stability of gluten free sweet biscuit elaborated with rice bran, broken rice and okara. *Food Science and Technology*, 36(2), 296–303. https://doi.org/10.1590/1678-457X.0083

Thompson, T., Dennis, M., Higgins, L.A., Lee, A.R., Sharrett, M.K. (2005). Gluten-free diet survey: are Americans with celiac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *Journal of Human Nutrition and Dietetics*, 18, 163-169. https://doi.org/10.1111/j.1365277X.2005.00607.x

Tibbetts, S.M., Milley, J.E., Lall, S.P. (2015). Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. *Journal of Applied Phycology*, 27, 1109–1119. https://doi.org/10.1007/s10811-014-0428-x

Torres, M.D., Chenlo, F., Moreira, R. (2018). Rheological effect of gelatinisation using different temperature-time conditions on potato starch dispersions: mechanical characterisation of the obtained gels. *Food and Bioprocess Technology*, 11(1), 132–140. https://doi.org/10.1007/s11947-017-2000-6

Torres, M.D., Fradinho, P., Rodríguez, P., Falqué, E., Santos, V., Domínguez, H. (2020). Biorefinery concept for discarded potatoes: Recovery of starch and bioactive compounds. *Journal of Food Engineering*, 275, 109886. https://doi.org/10.1016/j.jfoodeng.2019.109886

Turrini, F., Zunin, P., Catena, S., Villa, C., Alfei, S., Boggia, R. (2019). Traditional or hydro-diffusion and gravity microwave coupled with ultrasound as green technologies for the valorization of pomegranate external peels. *Food and Bioproducts Processing,* 117, 30–37. https://doi.org/10.1016/j.fbp.2019.06.014

van der Spiegel, M., Noordam, M.Y., van der Fels-Klerx, H.J. (2019). Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. *Comprehensive Reviews in Food Science and Food Safety*, 12, 662-678. https://doi.org/10.1111/1541-4337.12032

White, W.L., Wilson, P. (2015). World seaweed utilization. In: B.K. Tiwari, D.J. Troy (Eds.), *Seaweed Sustainability - Food and Non-Food Applications*, Academic Press, London, pp. 7-25.

Williams, L.A. (2019). Innova Market Insights Top Trends for 2020. *Prepared Foods,* 188, I.12 URL: https://www.preparedfoods.com/articles/123130-innova-market-insights-top-trends-for-2020

Xie, F., Zhang, W., Lan, X., Gong, S., Wu, J., Wang, Z. (2017). Physicochemical properties and structural characteristics of soluble dietary fibers from yellow and purple fleshed potatoes by-product. *International Journal of Food Properties*, 20(3), S2939-S2949. https://doi.org/10.1080/10942912.2017.1387557

Chapter 2. Functional and Thermorheological Properties of Rice Flour Gels for Gluten-Free Pasta Applications

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In Figure B the schematic procedure of chapter 2 is presented.

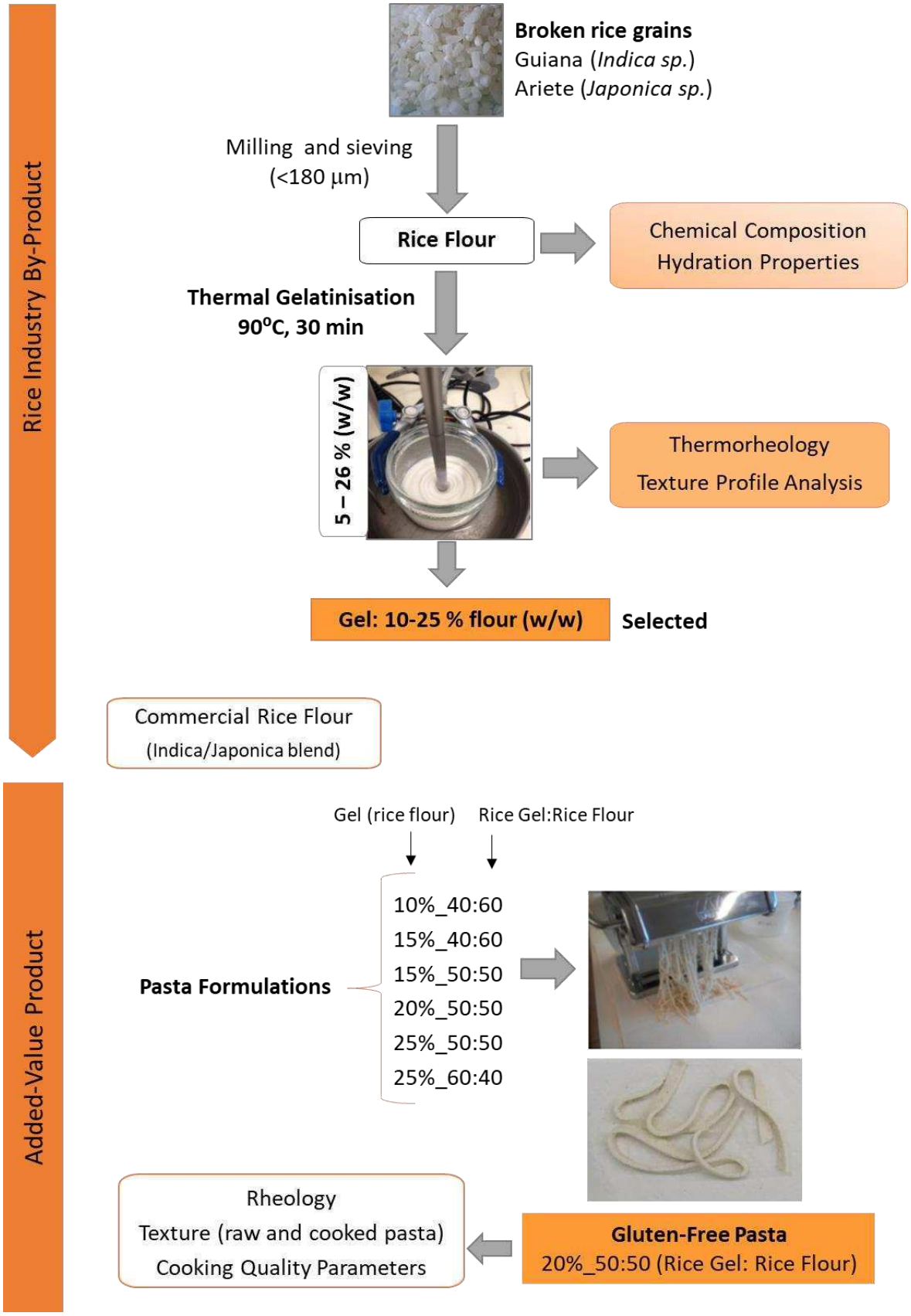

Figure B. Schematic procedure of chapter 2.

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Original article

Functional and thermorheological properties of rice flour gels for gluten-free pasta applications

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Summary Based on the European Commission directives on circular economy, this work explores the functional and thermorheological properties of rice flour from broken kernels, a by-product from the rice industry, in order to evaluate its possible use in food applications, back into the value chain. Chemical and hydration properties of two rice varieties (Guiana – *Indica* spp, Ariete – *Japonica* spp) were accessed, as well as the impact of the rice variety on the texture and viscoelasticity of rice flour gels (6–26%). Both rice varieties presented statistically different physicochemical properties (protein, lipid and amylose contents), and hydration properties that support the distinct texture and rheology behaviour of gels obtained. Rheology results suggested the formation of a gel-network structure with high frequency dependence, especially at lower rice concentrations. Texture results suggest that both rice varieties could be suited for development of gluten-free products that require pre-gelatinised starch, such as pasta. The effect of gelatinised rice flour (10–25%) and gelatinised flour/rice flour ratio (40:60, 50:50, 60:40) on the physical properties and cooking quality of pasta were assessed. Better overall cooking quality and texture properties were obtained for the formulation with 20% gelatinised rice flour and 50:50 rice gel:rice flour ratio.

Keywords Amylose, broken rice, gelatinization, rheology, swelling power, waste valorisation.

Introduction

Rice is a major cereal crop worldwide and one of the three important commodities in terms of food security. European countries are responsible for the production of 4.5 million ton of paddy rice, accounting for a self-sufficiency rate of about 65%. Although Portugal produces only 6% of the total rice produced in Europe, the portuguese are the top European rice consumers, with around 16 kg per capita per year (INE, 2016). Most of the rice (*Oryza sativa*) produced in Portugal is botanically classified as ssp. *Japonica*, commercially known as *carolino*, very well adapted to portuguese environmental conditions and traditionally used in culinary preparations. However, *Indica* varieties have a high expression in terms of consumption, due to the demand for exotic rices (e.g. basmati, thai) and pre-prepared convenient meals. With a total production of about 190 000 tons of paddy rice, it only covers 70–75% of Portugal needs but it is self-sufficient in *carolino* rice consumption.

In recent years, a growing interest to maximise the uses of the agricultural by-products for different purposes has been observed. During rice de-husking and polishing, considerable quantities of by-products are produced, including broken rice, which accounts for about 15% of the milling rice. This by-product is an important raw material in fermentation industries such as breweries and distilleries. Nevertheless, it can also be further milled into flour and used to develop gluten-free added-value foods . Several studies have been published on the development of sweet cookies (Tavares *et al*., 2016), snacks (Paiva *et al*., 2012), syrup (Spinosa *et al*., 2016) and noodles (Ahmed *et al*., 2015) using broken rice flour.

The development of gluten-free foods is on today's agenda. Rice flour is the most suitable commodity for bakery applications due to its bland taste, white colour, digestibility and hypoallergenic properties. However, rice protein lacks the functionality of gluten to promote a viscoelastic dough structure. Therefore, bakery product's properties need to be modified by appropriate structure and texture forming ingredients or additives, including various hydrocolloids (Lazaridou *et al*., 2007; Crockett *et al*., 2011), proteins (Sozer, 2009) and emulsifiers (Lai, 2001).

The approach proposed in the present work is to optimise the specific thermal properties of starch to improve

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dough viscoelastic characteristics, as reported by Padalino *et al*. (2013) and Marti *et al*. (2011) to produce gluten-free pasta with suitable technological quality.

Flours from different rice varieties differ in the gelatinisation behaviour and viscoelastic properties. Since starch is the main component of rice flour and greatly responsible for the cooking quality of rice, most studies (e.g. Singh *et al*., 2006, 2007; Kong *et al*., 2015) are focused on rice starches physicochemical and thermal properties, mainly from *Indica* rice varieties. However, other components present in the rice flour (protein, lipid and non-starch polysaccharides) take part in the gel formation, either facilitating or hindering the gelatinisation/retrogradation processes (Puncha-arnon & Uttapap, 2013). During gelatinisation process, an amylose-lipid complex is formed, restricting the expansion of starch granules during gelatinisation or retarding amylopectin retrogradation (Tester & Morrison, 1990; Yu *et al*., 2012). Several studies have been conducted with rice starches isolated from rice flour (e.g. Kong *et al*., 2015; Jang *et al*., 2016), however, there are few studies available about the thermorheological properties of rice flour (Torres *et al*., 2014). The present approach is focused on using broken rice by-product from the rice industry, following the European Commission strategy on Circular Economy, transforming wastes into high value-added products, and increasing globalised market for secondary raw materials (European Commission, 2015).

The main objective of the present work is to analyse the physicochemical, hydration and thermorheological properties of rice flour gels produced from broken rice's milling of Ariete, the most common portuguese *Japonica* variety, and Guiana rice (*Indica* variety), in order to evaluate their application in the development of gluten-free products, namely fresh pasta.

Materials and methods

Raw materials

Broken rice kernels from *O. sativa* ssp. *Indica* and *Japonica*, namely from Guiana and Ariete varieties, respectively, were provided by portuguese rice company Novarroz – Produtos Alimentares SA. Both rice varieties were milled and sieved to a size particle less than 180 μm in a Pulverisette 14 Premium (Fritsch) and kept at room temperature in a closed container protected from light.

Commercial rice flour Ceifeira (Dacsa Atlantic SA, lot 2545/17) was also used for the development of the gluten-free pasta.

Chemical composition of rice flours

Rice flours were analysed for its moisture according to AACC method 44-15.02 (AACC International, 1999a) and ash NP 518 (1986), based on gravimetric methods. Crude protein was determined by the micro Kjeldhal method according to the ISO20483 (2006) official method for cereals and pulses. The determined total nitrogen content was multiplied by a conversion factor of 5.95 (FAO, 2003) to obtain the flour's protein content. The rice flour's fat content was determined according to the procedure used for cereals and derived products in the Portuguese standard method NP 4168 (1991). Carbohydrate content was calculated by difference to 100% of main constituents (moisture, ash, protein and fat).

Amylose content and amylose/amylopectin ratio of rice samples were determined enzymatically using the K-AMYL 06/2015 Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Amylose is expressed as a percentage of total starch. All chemical analyses were carried out at least in triplicate and expressed as the mean value ± standard deviation.

Hydration properties of rice flours

The swelling power (SP) of Guiana and Ariete rice flours was determined using the method developed by Leach *et al*. (1959) for starches, with slight modifications. This method involves the suspension of rice flour (w_{sample}) in excess water, with gentle stirring to keep it in suspension, followed by incubation at 20, 50, 70 and 90 °C (within the range of temperatures involved in the starch gelatinisation process), for 30 min and centrifugation at 10 000 ***g*** for 10 min. Swelling power was determined from the weight of the sediment ($w_{sediment}$) according to eqn (1):

$$\mathrm{SP\,(g\ g^{-1}, dry\ basis)} = w_{sediment}/(w_{sample} - w_{residue}) \quad (1)$$

The water absorption index (WAI) and solubility (S) of rice flours were assessed by the method described by Anderson (1982), applying the same range of temperatures as for SP. Both parameters were determined according to eqns (2) and (3):

$$\mathrm{WAI\,(g\ g^{-1}, dry\ basis)} = w_{sediment}/w_{sample} \quad (2)$$

$$\mathrm{S\,(\%, dry\ basis)} = (w_{residue}/w_{sample}) \times 100 \quad (3)$$

Rice flour gel preparation

Rice flour suspensions from Guiana and Ariete varieties were prepared, ranging from 4.5% to 26.0% (w/w, dry basis). Flour was dispersed in water, under mechanical stirring (IKA Labortechnik, EURO-STD, Staufen, Germany) at 350 r.p.m. and heated at 90 °C during 30 min, in a water bath, according to previously optimised conditions (Torres *et al*., 2014). The mixtures were poured into glass containers (35 mm height, 32 mm diameter) and left at 5 °C for 24 h to ensure full maturation.

Pasta preparation

In this study, commercial rice flour made from the milling of broken kernels from *Indica* and *Japonica* varieties was used. The flour composition in terms of rice varieties and their proportion was not provided by the manufacturer.

To assess the optimum pasta formulation, rice flour gel (10–25% d.b) was prepared according to procedure previously described. After cooling, rice flour gel was mixed with rice flour at 40:60, 50:50, 60:40 ratios, in a food processor (Bimby TM31; Vorwerk, Wuppertal, Germany) for 3 min at room temperature. Then, the dough was covered in aluminium foil and allowed to equilibrate for 15 min at 25 °C in an air oven, before measurements. Then, the dough was sheeted (2 mm diameter) and laminated as tagliatelle using a bench top pasta machine (Atlas 150, Marcato, Italy).

Rheology measurements

Rice flour suspensions (6–26%, w/w, d.b) from Ariete and Guiana varieties were dispersed under magnetic stirring (200 r.p.m.) at room temperature for 5 min, and immediately poured into the bottom plate of a 35 mm serrated parallel plate sensor (PP35) on the rheometer (MARS III; Haake, Karlsruhe, Germany), to promote gelatinisation *in situ*, avoiding further perturbations of the matrix. The temperature control was performed us Haake ing an UTC-Peltier system and the gap was set at 0.5 mm, according to Torres *et al.* (2014). Edges of samples were coated with liquid paraffin, to prevent moisture losses during tests.

Stress sweep tests were conducted on suspensions and on samples submitted to thermal treatment to ensure that all measurements were carried out within the viscoelastic region. Temperature, time and frequency sweep tests were performed inside this region at 1 Hz. Aqueous flour suspensions were held 5 min at 20 °C, between the plates, before testing. Small amplitude oscillatory shear measurements (SAOS) were performed to study the viscoelastic behaviour of the samples. First, the suspensions were heated from 20 to 90 °C at 2 °C min^{-1} and time sweep tests were conducted at 90 °C for 30 min. After thermal treatment, samples were cooled down to 5 °C at 2 °C min^{-1}. The maturation was performed at this temperature, during 30 min at 1 Hz, followed by the frequency sweep at 5 °C, with oscillation frequencies over the range 0.01–100 Hz. Each formulation was tested at least in duplicate.

The viscoelastic behaviour of dough formulations was performed in a controlled stress rheometer (MARS III; Haake) coupled with a UTC – Peltier system, using a serrated parallel plate system with 20 mm diameter and 2 mm gap. Frequency sweep tests at 20 °C were performed from 0.01 to 100 Hz, within the linear viscoelastic region. Each formulation was tested at least in triplicate.

Instrumental texture analysis

The texture profile analysis (TPA) of all rice flour gels and doughs was performed using a TA-XTplus Texture Analyser (Stable Micro Systems, Godalming, UK) in penetration mode, using a 10 mm acrylic probe.

The dough was moulded in acrylic discs (61.5 mm diameter and 18 mm height) and rested for 15 min before the probe plunged 8 mm at 1 mm s^{-1}. On the other hand, the gels were let to equilibrate at 20 °C for 30 min before a 15 mm perforation at 1 mm s^{-1}.

From the force vs. time texturograms, the parameters which discriminate the sample's texture were firmness and adhesiveness. Firmness (N) was considered as the maximum resistance to the penetration and was calculated as the height of the force peak. Adhesiveness ($-N$.s) is a characteristic of sticky materials and can be defined as the resistance of the material when the probe is recessing. This parameter is recorded as the negative area of the first cycle (Bourne, 2002). These determinations were conducted at 20 ± 1 °C in a temperature controlled room and were replicated at least eight times.

The firmness of cooked pasta samples prepared according to 2.5 was measured following AACC method 66-50.01 (AACC International, 1999b). Pasta firmness was determined by measuring the cutting force required to cut three cooked tagliatelle strips using a blade set with guillotine (HDP/BSG) that cut the sample at 0.17 mm s^{-1}. The thickness of the samples was measured to determine the cutting distance of each sample: 1.5–2.8 mm. Each formulation was replicated at least eight times.

Pasta stickiness is defined as the maximum peak force required to separate the probe from the sample surface (peak height) and the area under the peak as the work of adhesion Smewing (2009). Three tagliatelle strips were centrally aligned under a circular probe (44 mm diameter) on a raised platform and were retained within a circular slot (48 mm diameter) made in a base plate. The samples were compressed for 2 s with an applied force of 9.807 N at 0.5 mm s^{-1}. At least ten replicates were performed of each pasta formulation. The precision of the stickiness measurement decreases as elapsed time increases. Therefore, the time for stickiness measurement was set at 15 min after draining.

Cooking quality evaluation of pasta

The optimum cooking time was defined empirically by cooking the tagliatelle samples in boiling distilled water for 1 min. The swelling index (Padalino *et al.*, 2013) of cooked pasta was determined by weighing 10 g of the sample, before and after cooking, and then

drying it at 103 ± 2 °C until constant weight, and is determined by eqn (4).

$$\text{Swelling}\,(\text{mL g}^{-1}) = (\rho_{\text{water}} \times V_{\text{water absorbed during cooking}})/m_{\text{dry sample}} \quad (4)$$

Pasta water absorption is defined as the weight increase of pasta before and after cooking, and was determined as percent weight gain with respect to the weight of uncooked pasta. The samples were cooked, rinsed with water and allowed to drain for 5 min. The water absorption was then determined by eqn (5).

$$\text{Water absorption}\,(\%) = [(m_{\text{cooked sample}} - m_{\text{raw sample}})/m_{\text{raw sample}}] \times 100 \quad (5)$$

Cooking loss (Zhu *et al*., 2010) is defined by the amount of solids lost into the cooking water, and was determined by evaporating the 100 mL of water used for cooking 10 g of dough, and is expressed according to eqn (6).

$$\text{Cooking loss}\,(\%) = (m_{\text{residue after evaporation}}/m_{\text{sample}}) \times 100 \quad (6)$$

At least three measurements were performed for each analysis.

Statistical analysis

Experimental data were analysed using Statistica 10 (StatSoft Inc, Tulsa, OK, USA) by means of *t*-test and analysis of variance (one-way ANOVA) using the *Post Hoc* Comparison Tukey test, with 95% confidence ($P < 0.05$). Pearson correlation analysis was also conducted ($P < 0.05$) to determine the relationships between the physicochemical and hydration properties of the samples. To assess possible relationships between protein content, lipid content, SP (at 90 °C) and amylose (dependent variable) content of rice a multiple linear regression was performed.

Results and discussion

Chemical composition of rice flours

The chemical composition of rice samples is presented in Table 1.

As expected, carbohydrates are the main constituent of rice flour. In the traditional wheat semolina pasta, proteins play an important role in structure building through the establishment of a gluten network, and starch granules act as inner fillers. However, in a gluten-free matrix, starch assumes the major responsibility for functional properties, particularly its amylose content. According to the classification of rice based on amylose content (Juliano, 1998), Ariete (*Japonica*) variety is ranked as low amylose rice (10–20% amylose) and Guiana (*Indica*) is intermediate amylose (20–25% amylose). Other authors have reported amylose values between 4.1–16.4% (Singh *et al*., 2006) and 18.3–27.9% (Mariotti *et al*., 2009) for *Indica* rice varieties; and 10.5% for Giza (*Japonica*) (Ahmed *et al*., 2015) and 19.9% *Japonica* (Singh *et al*., 2000). Santos *et al*. (2013) reported amylose values between 9.4% and 15.2% for portuguese *Japonica* varieties. According to the results from Table 1, amylose content is highly dependent on the rice variety, and significantly higher ($P < 0.05$) for Guiana rice. The differences in physicochemical parameters of both rice varieties, mainly amylose content, could support the different technological behaviour of these rice varieties, as discussed later on.

Protein content is higher in Guiana (*Indica*) than in Ariete (*Japonica*) and both values are similar to those found by Singh *et al*. (2000) for other rice varieties. Most studies dealing with starch pasting properties are performed with isolated starch with residual protein content. Since protein content has an important impact on pasting properties, it is natural that the high protein values could help to explain the possible differences in the behaviour of the studied rice gels, when compared to gels obtained from isolated starch.

Hydration properties

The swelling index is an indicator of the water absorbed by the flour (mainly by starch and proteins) during cooking, which will be used for the gelatinisation of starch and the hydration of protein. The hydration properties of rice flour (Fig. 1) are related to physicochemical characteristics such as amylose and protein contents, and consequently to the gel formation (Lai, 2001), an important property in the food industry to define the range of application of the biopolymers.

Table 1 Chemical composition of Guiana and Ariete rice flours

Rice sample	Protein % (w/w, d.b)	Lipids* % (w/w, d.b)	Ash % (w/w, d.b)	Carbohydrates % (w/w, d.b)	Moisture (% w/w)	Amylose* (% w/w)	AM/AP
Guiana	7.4 ± 0.4	1.8 ± 0.1	0.6 ± 0.0	89.7	12.5 ± 0.1	22.4 ± 1.5	1/3.5
Ariete	6.4 ± 0.5	2.8 ± 0.7	0.6 ± 0.0	90.2	13.1 ± 0.2	16.4 ± 1.6	1/5.1

AM/AP, amylose/amylopectin ratio.
Samples marked with * showed significant differences ($P < 0.05$, *t*-test) for each parameter.

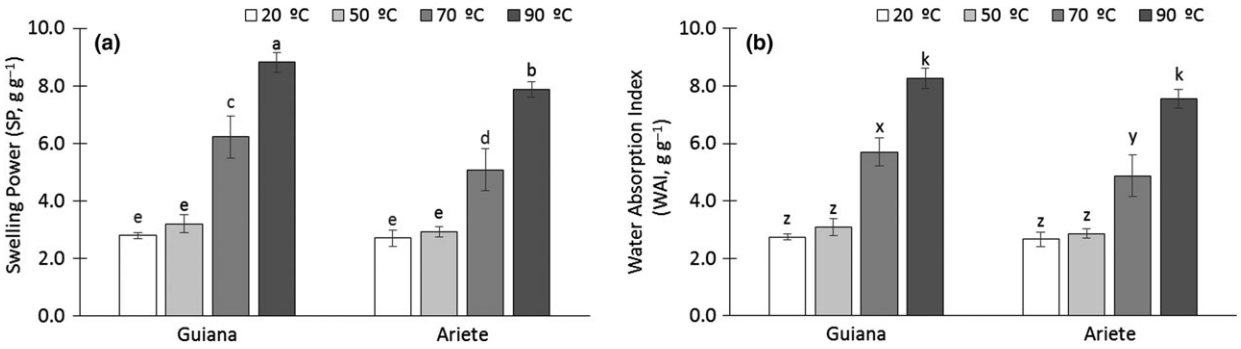

Figure 1 Impact of temperature of the Guiana (*Indica*) and Ariete (*Japonica*) rice flours on swelling power (a) and water absorption index (b). Samples marked with different letters showed significant differences ($P < 0.05$, one-way ANOVA *post-hoc* Tukey test) for each parameter.

Both rice varieties exhibited different gelatinisation behaviour, since at 20 °C (room temperature) there is no significant difference ($P < 0.05$) in SP, but at 70 and 90 °C, corresponding to the gelatinisation process, Guiana (*Indica*) exhibits significantly ($P < 0.05$) higher SP values. Differences in SP can resulted from differences in amylose content, viscosity pattern and weak internal organisation as a consequence of negatively charged phosphate groups within the rice starch granules (Singh *et al.*, 2006). Water absorption results from both rice varieties showed that only at 70 °C Guiana is significant ($P < 0.05$) more prone than Ariete to absorb water. Flours with high water absorption should have more hydrophilic constituents, such as polysaccharides (Kaushal *et al.*, 2012). Since the carbohydrate content is similar for both samples, the results can be explained by the amylose content of the studied samples.

The swelling behaviour of cereal starch is related to its pasting and rheological properties. This parameter has been reported as a property mainly related with the amylopectin content. Ariete presented a higher amylopectin (Table 1) content but lower SP than Guiana (at 70 °C) and this can be explained by its significantly lower amylose content (Ariete: 16.4%, Guiana: 22.4%). Although amylopectin swells to a greater extent along with protein and lipids as individual components, in combination with amylose there is a resistance to swelling (Fabian *et al.*, 2011). Amylose acts both as a diluent and as an inhibitor of swelling of the starch granules, especially in the presence of lipids (Tester & Morrison, 1990). In fact, amylose and lipid content are strongly negatively correlated ($r = -0.948$, $P < 0.05$).

The results of the multiple linear regression model were significant ($P < 0.001$, adj $R^2 = 0.999$) indicating that approximately 99.9% of the variance in the amylose results is explained by protein, lipids and SP (at 90 °C) according to the following correlation expression (7):

$$\text{Amylose} = 35.52 + 2.96\,\text{Protein} - 4.65\,\text{Lipid} - 2.71\,\text{SP} \quad (7)$$

As shown in Fig. 1a, SP of both rice flours increased with temperature, and have a steeper increase near the gelatinisation temperature. WAI (Fig. 1b) presents a similar pattern to SP. Guiana rice flour shows higher values in both parameters.

These results show a temperature-dependency of both swelling and WAI for both rice varieties, following polynomial eqns (8–11).

$$\text{SP}_{\text{Gu}} = 0.001T^2 - 0.070T + 3.610, R^2 = 1 \quad (8)$$

$$\text{SP}_{\text{Ar}} = 0.002T^2 - 0.122T + 4.447, R^2 = 0.999 \quad (9)$$

$$\text{WAI}_{\text{Gu}} = 0.001T^2 - 0.077T + 3.640, R^2 = 0.987 \quad (10)$$

$$\text{WAI}_{\text{Ar}} = 0.002T^2 - 0.091T + 3.857, R^2 = 0.997 \quad (11)$$

There was a significant correlation between SP and WAI (eqns 12 and 13) which is often reported by other authors (Wang & Seib, 1996; Peries *et al.*, 2016).

$$\text{SP}_{\text{Gu}} = -0.014\,\text{WAI}^2 + 1.179\,\text{WAI} - 0.292, \text{R}^2 = 1 \quad (12)$$

$$\text{SP}_{\text{Ar}} = -0.010\,\text{WAI}^2 + 1.182\,\text{WAI} - 0.347, \text{R}^2 = 1 \quad (13)$$

As expected, solubility (S) results increased with temperature, following a polynomial relation (eqns 14 and 15):

$$S_{\text{Gu}} = 0.001T^2 + 0.027T + 0.937, R^2 = 0.971 \quad (14)$$

$$S_{\text{Ar}} = 0.002T^2 - 0.077T + 2.457, R^2 = 0.998 \quad (15)$$

A strong positive correlation ($r = 0.965$, $P < 0.05$) was also found between solubility (at 50 °C) values and amylose content, since amylose linear chain is more water soluble than amylopectin. In this sense, a

flour with higher amylose content should be more easily used for making pre-gelatinised flours, which would of great interest for pasta making.

Rheology characterisation of rice gels

The empirical rheology method performed by the amylograph is the standard test accepted for determination of the gelatinisation properties of flour/starch. However, several authors (Singh *et al*., 2006; Mariotti *et al*., 2009) have been using fundamental rheology methods since they use smaller size samples, better precision and the ability to study different temperature profiles (Pojić *et al*., 2013).

As expected for a gel, an increase in the linear elastic modulus with rice flour concentration is observed (Fig. 2), which reflects an increase in the gel structure level as more gel linkages are formed. As the temperature increases, a sharp increase in G' is observed, which corresponds to the gelatinisation temperature range. For both rice varieties starch gelatinisation occurs between 52.6 and 78.2 °C and is dependent on rice content, since this parameter increases with the increase of rice concentration. For lower rice concentrations, there is a steeper increase in G' with heating temperature, while for rice flour concentrations above 17.5%, the temperature does not have such a marked influence on G'. Moreover, for Ariete rice variety (Fig. 2b) this behaviour is noticed even at 9% rice concentration.

Since water influences both the flexibility and the molecular mobility of starch molecules, in a more concentrated gel, water molecules seem to have less mobility as they are close packed and more extensively hydrogen bonded to relatively large and immobile starch molecules (Lu *et al*., 2011). At these high rice flour concentrations, there is not enough available water for the swelling of all starch granules. So, as a consequence, amylose is not completely leached and no steep increase in the G' is observed.

Gelatinisation temperature is not only related to amylose content, it also depends on granular architecture, molecular weight distribution and amylopectin fine structure (Sodhi & Singh, 2003). For all rice concentrations tested, Ariete rice variety presents higher gelatinisation temperature than Guiana. Although this study focus only two rice varieties, Mariotti *et al*. (2009) also reported a significative correlation between gelatinisation temperature and amylose content.

Although the gelatinisation temperature varies with rice concentration, Peak Temperature is almost identical (85.2–87.6 °C) for all concentrations at both rice flours. Similar pattern but different temperature ranges were obtained by Spigno & De Faveri (2004) for starch contents between 10% and 80%.

During the cooling process (data not shown), an increase of viscoelastic parameter G' with rice flour content is noticed as expected, meaning that a slight reinforcement due to more linkages between molecules of the gel structure is observed on cooking. This behaviour is in agreement with the formation of a continuous network by the solubilised amylose molecules that link swollen starch granules by hydrogen bonds. The maturation kinetics (data not shown) of all rice concentrations showed a rapid gel maturation (G' and G'' remain constant) just after a few minutes of maturation. This feature brings a technological advantage for future use of pre-gelatinised rice flour in gluten-free products.

The rheology behaviour of Guiana and Ariete gels after maturation is depicted in Fig. 3.

For all rice concentrations studied from both rice varieties, G' is always higher than G'', indicating the existence of a three-dimensional network formed by carbohydrate and protein macromolecules that trap the water molecules and starch granules. As expected for gel systems the structure is reinforced with intermolecular linkages with the increase of rice flour, therefore viscoelastic parameters increase with concentration. However, from 17.5% rice content there is

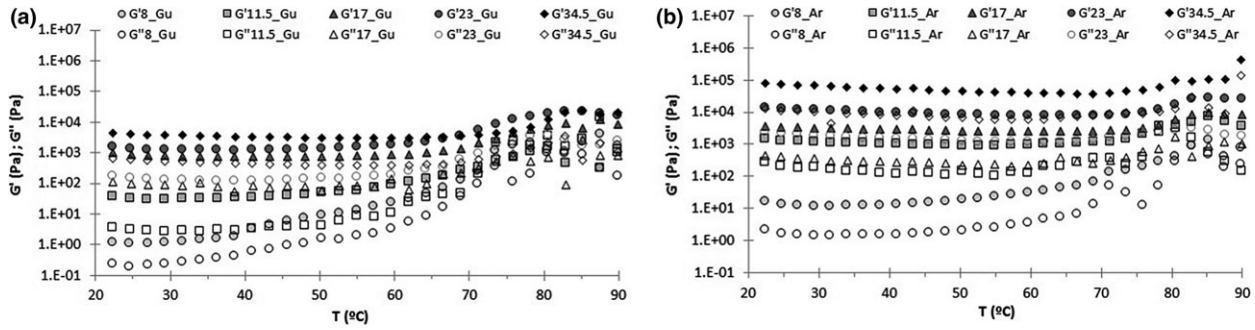

Figure 2 Evolution of viscoelastic moduli during heating (2 °C min^{-1}) of Guiana (a) and Ariete (b) rice flours at 6%, 9%, 13%, 17.5% and 26% rice content (closed symbols-G'; open symbols-G'').

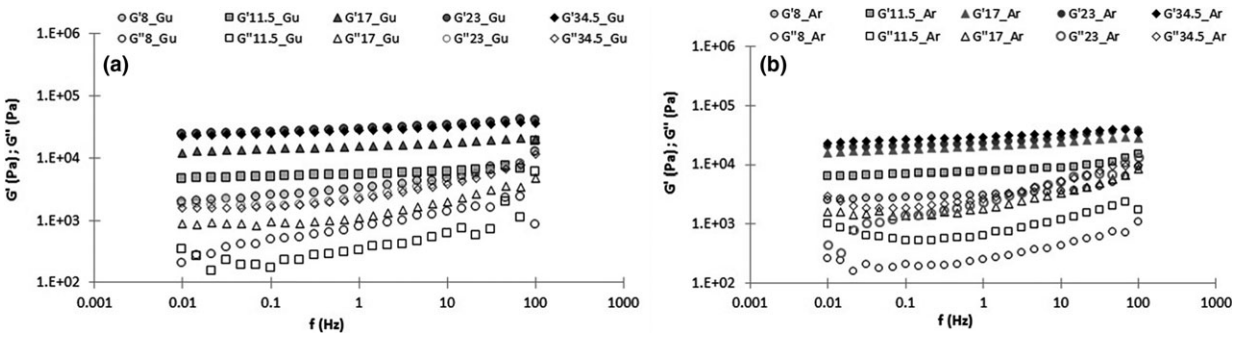

Figure 3 Mechanical spectra of (a) Guiana and (b) Ariete rice flour gels (closed symbols-G'; open symbols-G'') at different concentrations (6–26% w/w, d.b).

little increase in the viscoelastic moduli especially for Ariete variety. This phenomenon is related with close packing of the molecules due to the tight association between starch molecules in high starch concentrations as discussed earlier.

At low rice concentrations (6% and 9%) G'' tends to exhibit a minimum, which has been related to the formation of physical entanglements among polymeric molecules, that reinforce the three-dimensional network (Ferry, 1980). Especially at lower rice contents, both viscoelastic moduli increase with the frequency, with a stronger dependency for G''. This results in a weak gel-like behaviour of the rice pastes, as previously reported by Mariotti *et al.* (2009) for flours from several rice varieties.

Texture of rice flour pastes

For the suspension prepared with 4.5% (w/w) rice flour no gel formation was observed. Consequently, this concentration was not used for further studies. According to Lu *et al.* (2011) for starch concentrations lower than 5%, the leached-out amylose and swollen granules are not high enough to interact themselves to form a matrix. In the present study, 6% (w/w) is probably the critical gelation concentration.

The texture of rice flour pastes was evaluated by a TPA penetration test, and the resulting firmness and adhesiveness parameters were determined and presented in Fig. 4.

For both rice varieties, gel firmness and adhesiveness are nearly constant until 13% rice flour concentration. Texture results show that for rice content above this concentration, both texture parameters suffered a steeper increase ($P < 0.05$) associated with the reduced mobility of water molecules, as discussed previously. This could indicate a maximum rice flour concentration for future use in food applications, since high adhesiveness values bring technological constraints and undesirable features in pasta development. Therefore, 17.5% should be close to the maximum rice concentration usable, although other ingredients added in the pasta making process (e.g. other flours, hydrocolloids) would also contribute to the adhesiveness of the final product.

It is also observed that considering the same rice flour concentration in Guiana and Ariete, there is a statistical difference ($P < 0.05$) between firmness and adhesiveness values of the two rice varieties for 17.5% and 26.0% rice content, respectively. These results are in agreement with the ones reported by Lu *et al.* (2011) for different varieties of both rice subspecies. These results are consistent with the rheology study previously discussed in Section Rheology characterisation of rice gels.

As referred by Jang *et al.* (2016), these textural properties of starch gels are dependent on the amylose and protein contents. Pearson correlation was performed between physicochemical properties of flours and texture parameters of gels to verify if a correlation between these properties can be applied. In a review published by Marti & Pagani (2013) several studies are mentioned that report the high correlation between high amylose (25–33%) rice and the sensory acceptance of the pasta produced. Therefore, the ideal starch for gluten-free (GF) pasta products should have a marked tendency to retrograde: this property is useful to give rigidity to cooked pasta, and to reduce both the stickiness of the pasta surface and the cooking loss. However, other studies (e.g. Ahmed *et al.*, 2015) are in favour of low amylose content. A *Japonica* rice (Giza variety) was selected as the best for the development of gluten-free products due to its high water holding capacity, peak viscosity and above all low amylose (11.6%).

Optimisation of pasta formulation

As observed in this study, Guiana rice variety showed statistically ($P < 0.05$) higher amylose content than Ariete, which could be more suited for GF pasta applications. However, due to industrial constrains related with low market demand for rice flour, broken

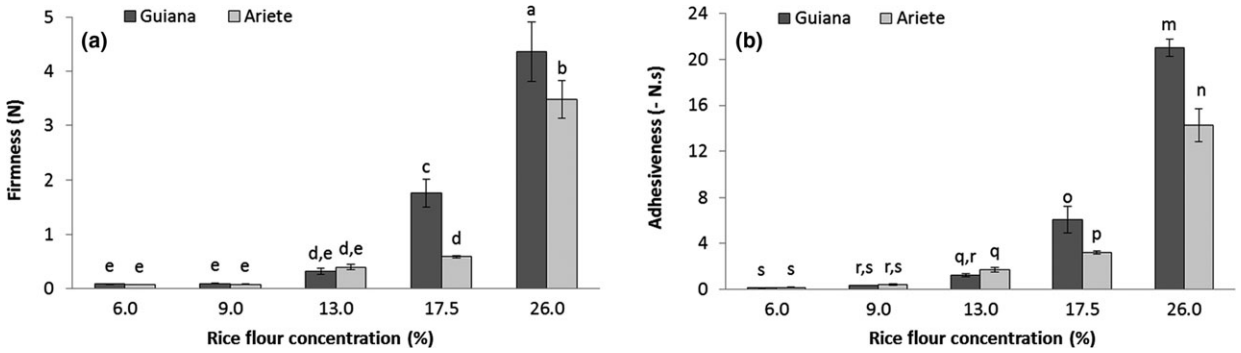

Figure 4 Texture parameters (a – firmness; b – adhesiveness) of rice gels (6–26% w/w, d.b). Different letters indicate significant differences ($P < 0.05$, one-way ANOVA *post-hoc* Tukey test) for the same parameter.

kernels are milled all together, independently of rice variety. Therefore, rice flour available in the portuguese market is a blend of both *Indica* and *Japonica* rice varieties, in random proportions.

For this reason, the optimisation of pasta formulation was performed using a commercial rice flour with the following chemical composition: 6.0 (±0.6)% protein, 0.8 (±0.1)% lipids, 11.5 (±1.7) % amylose, 1/7.7 AM/AP (amylose/amylopectin ratio).

Several formulations of rice pasta were tested according to the procedure described in Section Pasta preparation. However, not all of them are suitable for pasta formation (Fig. 5).

The optimisation of pasta formulation was performed taking into account the formulations 10%_40:60, 15%_40:60, 15%_50:50, 20%_50:50, 25%_50:50, 25%_60:40. Both dough and pasta made from these formulations were characterised in terms of their physical, rheological and cooking quality parameters. From a processing point of view, the best doughs which revealed to be less sticky and easier to handle, were: 20%_50:50 and 25%_50:50.

Physical properties of uncooked pasta dough

The rheological behaviour of dough formulations prepared with different proportions of rice gel is depicted in Fig. 6.

Viscoelastic behaviour of the doughs shows a weak gel-like structure, with a slight frequency dependence. For all gluten-free pasta analysed, a similar trend was observed regardless of dough composition.

To quantify the impact of different combinations of gelatinised rice flour and rice flour on the elastic modulus, the variation of G' with dough composition is represented in Fig. 7, at three frequency values (0.1, 1 and 10 Hz) obtained from the respective mechanical spectra. It is observed that a higher gel concentration leads to a more structured dough, whereas higher rice gel ratio leads to a less structured dough. This behaviour is consistent with the doughs texture profile (Fig. 8).

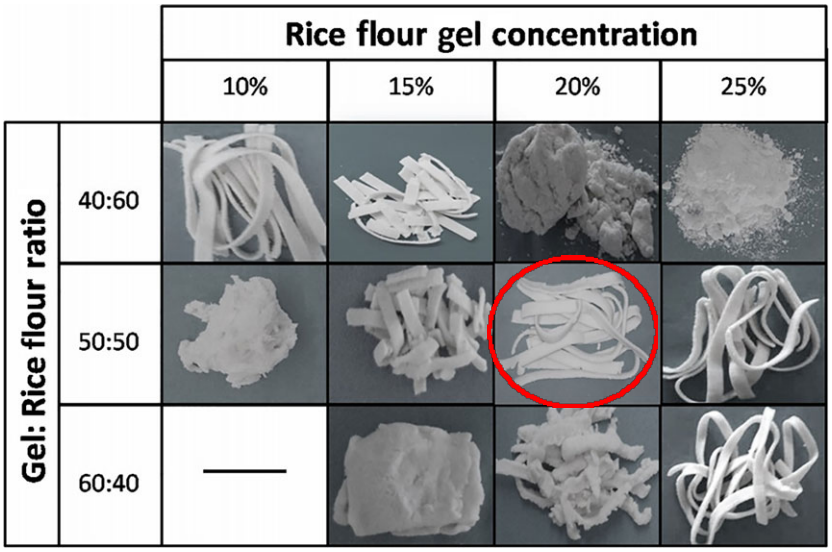

Figure 5 Dough formulations produced with 10–25% rice flour and different gel:rice flour ratios (40:60, 50:50 and 60:40). [Colour figure can be viewed at wileyonlinelibrary.com]

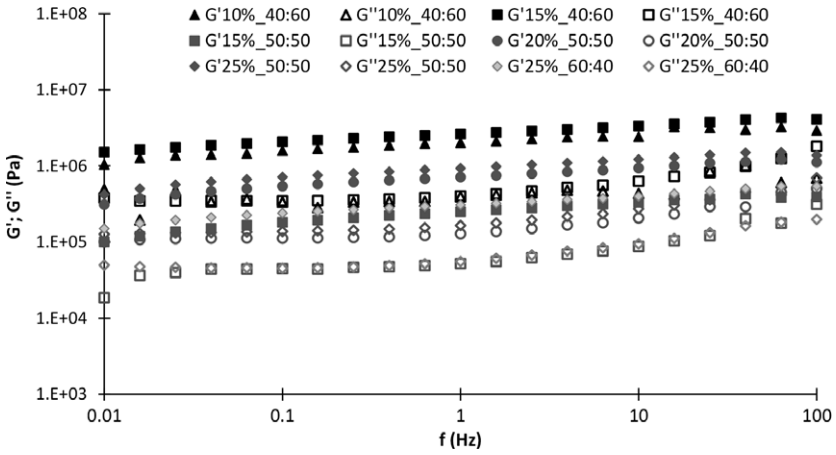

Figure 6 Mechanical spectra of dough formulations: 10%_40:60, 15%_40:60, 15%_50:50, 20%_50:50, 25%_50:50, 25%_60:40 (% rice flour gel_rice gel:rice flour ratio).

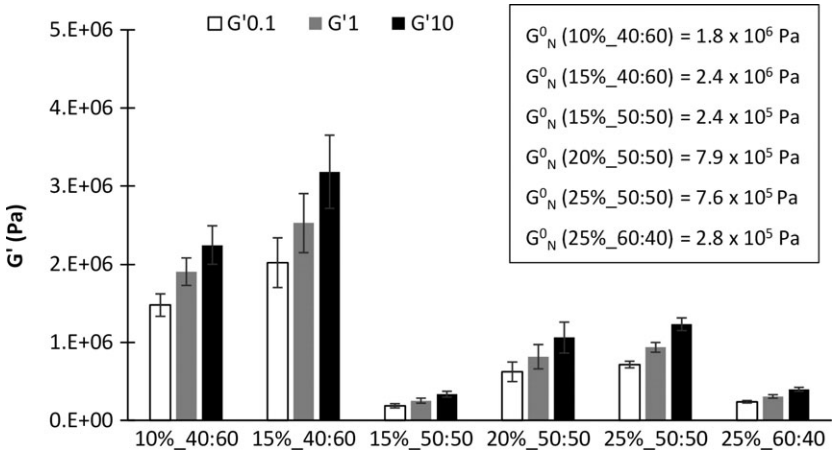

Figure 7 G' at 0.1 (□), 1 (□) and 10 Hz (■) of dough with 10–25% rice flour gel concentration and different gel:rice flour ratios (40:60, 50:50 and 60:40).

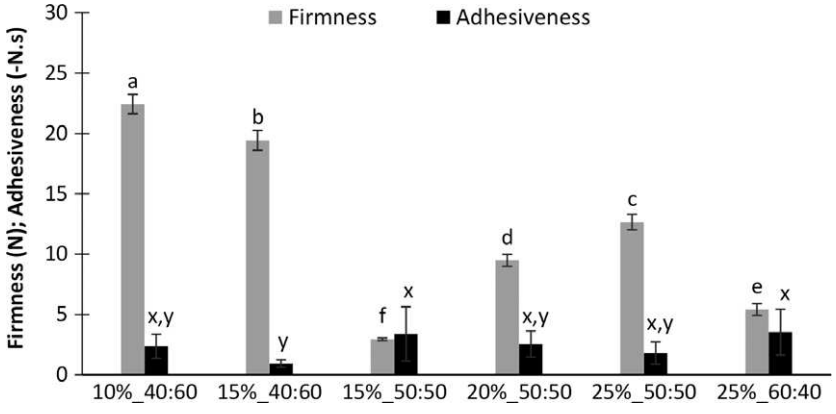

Figure 8 Texture parameters of dough formulations. Different letters indicate significant differences ($P < 0.05$, one-way ANOVA *post-hoc* Tukey test) for the same parameter.

In the texture tests all dough formulations showed statistically different firmness values. It is noteworthy that firmness is more dependent on the rice gel:flour ratio (40:60, 50:50, 60:40) than on rice flour concentration (10–25%). Considering the same rice gel:flour ratio (e.g. 50:50), a significant increase in firmness is observed for different rice gel concentrations (e.g. 15%, 20%, 25%). It is also observed that increasing gel:flour ratio, for the same gel concentration (25%_50:50 and 25%_60:40), a significantly decrease in firmness is observed.

Cooked pasta quality

The cooking behaviour, which is a critical step for quality perception of pasta, includes the evaluation parameters presented in Table 2.

Cooking loss is commonly used to predict the pasta cooking quality and is an indicator of the capability of the starch-protein matrix to retain its physical integrity during cooking. Traditionally, the loss of solids represents a measure of pasta quality, expressing its resistance to disintegration upon boiling: low amounts of solids into the cooking water indicate a high pasta cooking quality (Pagani *et al*., 2007). According to Schmiele *et al*. (2013) good quality wheat pasta should present a maximum cooking loss of 6%. However, unlike durum wheat pasta, due to the lack of gluten, the characteristics of GF pasta depend heavily upon the functional properties of the starch, since in the present study functions as almost the only structural network of the final GF product, since protein play a minor role due to its low content (6%) in the flour used.

Water absorption is related with the pasta yield after cooking (Zhu *et al*., 2010), and the swelling index is an indicator of the water uptake by starch and proteins during cooking, as a measure for gelatinisation and protein hydration (Padalino *et al*., 2013).

Comparing uncooked and cooked pasta, there is no correlation ($P < 0.05$) in firmness neither in adhesiveness values (Fig. 9). This can be explained considering that the uncooked dough is a partially gelatinised product, so the thermal modifications suffered by the starch during cooking are not so pronounced comparing to what happens in wheat pasta.

Using optimisation criteria for each parameter: cooking loss, stickiness and adhesiveness were minimised, while swelling, water absorption and firmness were maximised, the pasta with the best characteristics was the 20%_50:50.

Generally, the ideal starch for GF pasta products should have a marked tendency to retrograde, property generally observed in high amylose cereals and pulses, this assures good cooking behaviour in terms of texture and low cooking loss, even after prolonged cooking (Marti & Pagani, 2013). However, the commercial flour used in this study, with low amylose content, showed to be suitable for the production of a good quality GF pasta, with low stickiness and cooking losses.

Table 2 Cooking quality parameters of pasta formulations prepared with gelatinised rice flour

Pasta formulation	Swelling power (mL g^{-1})	Water absorption (%)	Cooking loss (%)
10%_40:60	0.79 ± 0.09^{b}	$47.76 \pm 5.43^{y,z}$	1.57 ± 0.38^{n}
15%_40:60	0.96 ± 0.05^{a}	55.74 ± 2.30^{x}	5.50 ± 0.47^{m}
15%_50:50	0.50 ± 0.05^{d}	26.92 ± 2.99^{w}	1.48 ± 0.30^{n}
20%_50:50	$0.73 \pm 0.01^{b,c}$	41.24 ± 0.32^{z}	1.05 ± 0.12^{n}
25%_50:50	$0.85 \pm 0.03^{a,b}$	$49.09 \pm 1.18^{x,y}$	1.60 ± 0.40^{n}
25%_60:40	0.64 ± 0.03^{c}	33.72 ± 1.82^{w}	1.40 ± 0.37^{n}

Samples marked with different letters, showed significant differences ($P < 0.05$, one-way ANOVA *post hoc* Tukey test) for each parameter.

Conclusions

The use of flour from broken rice kernels constitutes a valuable raw material with good gelling abilities for food applications. Based upon physicochemical and hydration properties, texture and rheology measurements of the respective gels, both Guiana and Ariete rice varieties presented suitable characteristics for food applications.

Using the present results for a specific food application like production of fresh gluten-free pasta, the maximum rice concentration for pasta making should be around 17.5%, although incorporation of other ingredients could interfere with this value. This critical concentration seems to be independent of the amylose content of rice (16.4% Ariete and 22.4% Guiana).

Based on cooking quality and texture parameters of all pasta formulations, the optimal formulation is the one with 20% rice gel and a 50:50 gel:flour ratio.

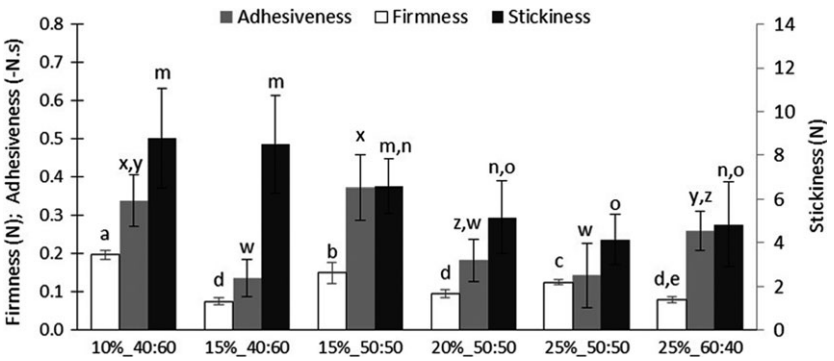

Figure 9 Texture parameters of cooked pasta formulations prepared with gelatinised rice flour. Different letters indicate significant differences ($P < 0.05$, one-way ANOVA *post-hoc* Tukey test) for the same parameter.

Since this product is designed for celiac consumers with a different sense of taste than of non-celiac, a sensory analysis should be performed with a consumer panel of celiac people.

Acknowledgments

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References

AACC International (1999a). *Approved Methods of Analysis*. 11th edn. Method 44-15.02 – Moisture: Air-Oven Methods. Reapproval November 3, 1999. St. Paul, MN: AACC International.

AACC International (1999b). *Approved Methods of Analysis*. 11th edn. Method 66-50.01 – Pasta and Noodle Cooking Quality. St. Paul, MN: AACC International.

Ahmed, J., Al-Jassar, S. & Thomas, L. (2015). A comparison in rheological, thermal, and structural properties between Indian Basmati and Egyptian Giza rice flour dispersions as influenced by particle size. *Food Hydrocolloids*, **48**, 72–83.

Anderson, R.A. (1982). Water absorption and solubility and amylograph characteristics of roll-cooked small grain products. *Cereal Chemistry*, **59**, 265–269.

Bourne, M. (2002). *Food Texture and Viscosity*. 2nd edn. London: Academic Press.

Crockett, R., Ie, P. & Vodovotz, Y. (2011). How do xanthan and hydroxypropyl methylcellulose individually affect the physicochemical properties in a model gluten-free dough? *Journal of Food Science*, **76**, E274–E282.

European Commission (2015). Closing the loop – an EU action plan for the circular economy. Brussels, COM(2015)614.

Fabian, C., Ayucitra, A., Ismadji, S. & Ju, Y.-H. (2011). Isolation and characterization of starch from defatted rice bran. *Journal of the Taiwan Institute of Chemical Engineers*, **42**, 86–91.

FAO (2003). *Food Energy – Methods of Analysis and Conversion Factors*. Pp. 93. FAO, Food and Nutrition Paper 77. Rome: Food and Agriculture Organization of the United Nations.

Ferry, J.D. (1980). *Viscoelastic Properties of Polymers*. 3rd edn. New York: John Wiley & Sons Inc.

INE (2016). Instituto Nacional de Estatística (Statistics Portugal). Available online at: https://www.ine.pt/xportal/xmain?xpgid=ine_main&xpid=INE

ISO20483 (2006). Cereals and pulses – determination of the nitrogen content and calculation of the crude protein content – Kjeldahl method. International Organization for Standardization.

Jang, E.-H., Su-Jin Lee, S.-J., Hong, J.-Y. *et al*. (2016). Correlation between physicochemical properties of japonica and indica rice starches. *LWT – Food Science and Technology*, **66**, 530–537.

Juliano, B.O. (1998). Varietal impact on rice quality. *Cereal Foods World*, **43**, 207–222.

Kaushal, P., Kumar, V. & Sharma, H.K. (2012). Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (*Colocasia esculenta*), rice (*Oryza sativa*) flour, pigeonpea (*Cajanus cajan*) flour and their blends. *LWT – Food Science and Technology*, **48**, 59–68.

Kong, X., Zhu, P., Sui, Z. & Bao, J. (2015). Physicochemical properties of starches from diverse rice cultivars varying in apparent amylose content and gelatinisation temperature combinations. *Food Chemistry*, **172**, 433–440.

Lai, H.M. (2001). Effects of rice properties and emulsifiers on the quality of rice pasta. *Journal of the Science of Food and Agriculture*, **82**, 203–216.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N. & Biliaderis, C.G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*, **79**, 1033–1047.

Leach, H.W., McCowen, I.D. & Schoch, T.J. (1959). Structure of the starch granule. I. Swelling and solubility patterns of various starches. *Cereal Chemistry*, **36**, 534–544.

Lu, S., Chen, J.-J., Chen, Y.-K., Lii, C.-Y., Lai, P. & Chen, H.-H. (2011). Water mobility, rheological and textural properties of rice starch gel. *Journal of Cereal Science*, **53**, 31–36.

Mariotti, M., Sinelli, N., Catenacci, F., Pagani, M.A. & Lucisano, M. (2009). Retrogradation behaviour of milled and brown rice pastes during ageing. *Journal of Cereal Science*, **49**, 171–177.

Marti, A. & Pagani, M.A. (2013). What can play the role of gluten in gluten free pasta? *Trends in Food Science and Technology*, **31**, 63–71.

Marti, A., Pagani, M.A. & Seethraraman, K. (2011). Understanding starch organisation in gluten-free pasta from rice flour. *Carbohydrate Polymers*, **84**, 1069–1074.

NP 4168 (1991). *Cereais e derivados – Determinação do teor de matéria gorda total*. Lisboa: Instituto Português da Qualidade.

NP 518 (1986). *Cereais e Leguminosas – Determinação do teor de cinza. Processo por incineração a 550 °C*. Lisboa: Instituto Português da Qualidade.

Padalino, L., Mastromatteo, M., De Vita, P., Ficco, D.B.M. & Del Nobile, M.A. (2013). Effects of hydrocolloids on chemical properties and cooking quality of gluten-free spaghetti. *International Journal of Food Science and Technology*, **48**, 972–983.

Pagani, M.A., Lucisano, M. & Mariotti, M. (2007). Traditional Italian products from wheat and other starchy flours. In: *Handbook of Food Products Manufacturing* (edited by Y.H. Hui). Pp. 327–388 Hoboken, NJ: John Wiley & Sons Inc.

Paiva, A.P., Barcelos, M.F.P., Pereira, J.A.R., Ferreira, E.B. & Ciabotti, S. (2012). Characterization of Food bars manufactured with agroindustrial by-products and waste. *Ciência e Agrotecnologia*, **36**, 333–340.

Peries, C.M., Wijesekara, K.B. & Navarathne, S.B. (2016). Effect of freezing and hot water soaking on the functional properties of rice flour derived from four Sri Lankan traditional rice varieties. *Procedia Food Science*, **6**, 68–72.

Pojić, M., Hadnađev, M. & Hadnađev, T. (2013). Gelatinization properties of wheat flour as determined by empirical and fundamental rheometric method. *European Food Research and Technology*, **237**, 299–307.

Puncha-arnon, S. & Uttapap, D. (2013). Rice starch vs. rice flour: differences in their properties when modified by heat–moisture treatment. *Carbohydrate Polymers*, **91**, 85–91.

Santos, T., Lemos, D., Brites, C., Guiné, R.P.F. & Correia, P.R. (2013). Contribuição para a caracterização de cultivares de arroz. Proceeding from the VII Congresso Ibérico de Agroingeniería y Ciencias Hortícolas, Ref. N°C0048: Madrid, Spain.

Schmiele, M., Jaekel, L.Z., Ishida, P.M.G., Chang, Y.K. & Steel, C.J. (2013). Massa alimentícia sem glúten com elevado teor proteico obtida por processo convencional. *Ciência Rural*, **43**, 908–914.

Singh, V., Okadome, H., Toyoshima, H., Isobe, S. & Ohtsubo, K. (2000). Thermal and physicochemical properties of rice grain, flour and starch. *Journal of Agriculture and Food Chemistry*, **48**, 2639–2647.

Singh, N., Kaur, L., Sandhu, K.S., Kaur, J. & Nishinari, K. (2006). Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocolloids*, **20**, 532–542.

Singh, N., Nakaura, Y., Inouchi, N. & Nishinari, K. (2007). Fine structure, thermal and viscoelastic properties of starches separated from Indica rice cultivars. *Starch/Stärke*, **59**, 10–20.

Smewing, J. (2009). Pasta-the invariable variables. *Food Science and Technology Today*, 1–4.

Sodhi, N.S. & Singh, N. (2003). Morphological, thermal and rheological properties of starches separated from rice cultivars grown in India. *Food Chemistry*, **80**, 99–108.

Sozer, N. (2009). Rheological properties of rice pasta dough supplemented with proteins and gums. *Food Hydrocolloids*, **23**, 849–855.

Spigno, G. & De Faveri, D.M. (2004). Gelatinization kinetics of rice starch studied by non-isothermal calorimetric technique: influence of extraction method, water concentration and heating rate. *Journal of Food Engineering*, **62**, 337–344.

Spinosa, W.A., Júnior, V.S., Galvan, D., Fiorio, J.L. & Gomez, R.J.H.C. (2016). Syrup production via enzymatic conversion of a byproduct (brokenrice) from rice industry. *Acta Scientiarum. Technology Maringá*, **38**, 13–22.

Tavares, B.O., Silva, E.P., Silva, V.S.N., Soares Júnior, M.S., Ida, E.I. & Damiani, C. (2016). Stability of gluten free sweet biscuit elaborated with rice bran, broken rice and okara. *Food Science and Technology*, Campinas, **36**, 296–303.

Tester, R.F. & Morrison, W. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, **67**, 551–557.

Torres, M.D., Fradinho, P., Raymundo, A. & Sousa, I. (2014). Thermorheological and textural behaviour of gluten-free gels obtained from chestnut and rice flours. *Food and Bioprocess Technology*, **7**, 1171–1182.

Wang, L. & Seib, P.A. (1996). Australian salt-noodle flours and their starches compared to US wheat flours and their starches. *Cereal Chemistry*, **73**, 167–175.

Yu, S., Ma, Y., Menager, L. & Sun, D.-W. (2012). Physicochemical properties of starch and flour from different rice cultivars. *Food and Bioprocess Technology*, **5**, 626–637.

Zhu, F., Cai, Y.-Z. & Corke, H. (2010). Evaluation of Asian salted noodles in the presence of *Amaranthus* betacyanin pigments. *Food Chemistry*, **118**, 663–669.

Chapter 3. *Psyllium* husk gel to reinforce structure of gluten-free pasta?

The work presented in this chapter was included in the publication:

- ***Research Paper 2***

  Fradinho, P., Soares, R., Niccolai, A., Sousa, I., Raymundo, A. (2020). *Psyllium husk gel to reinforce structure of gluten-free pasta? LWT - Food Science and Technology,* 131, 109787. https://doi.org/10.1016/j.lwt.2020.109787 (Impact Factor: 3.714, Q1)

In Figure C the schematic procedure of chapter 3 is presented.

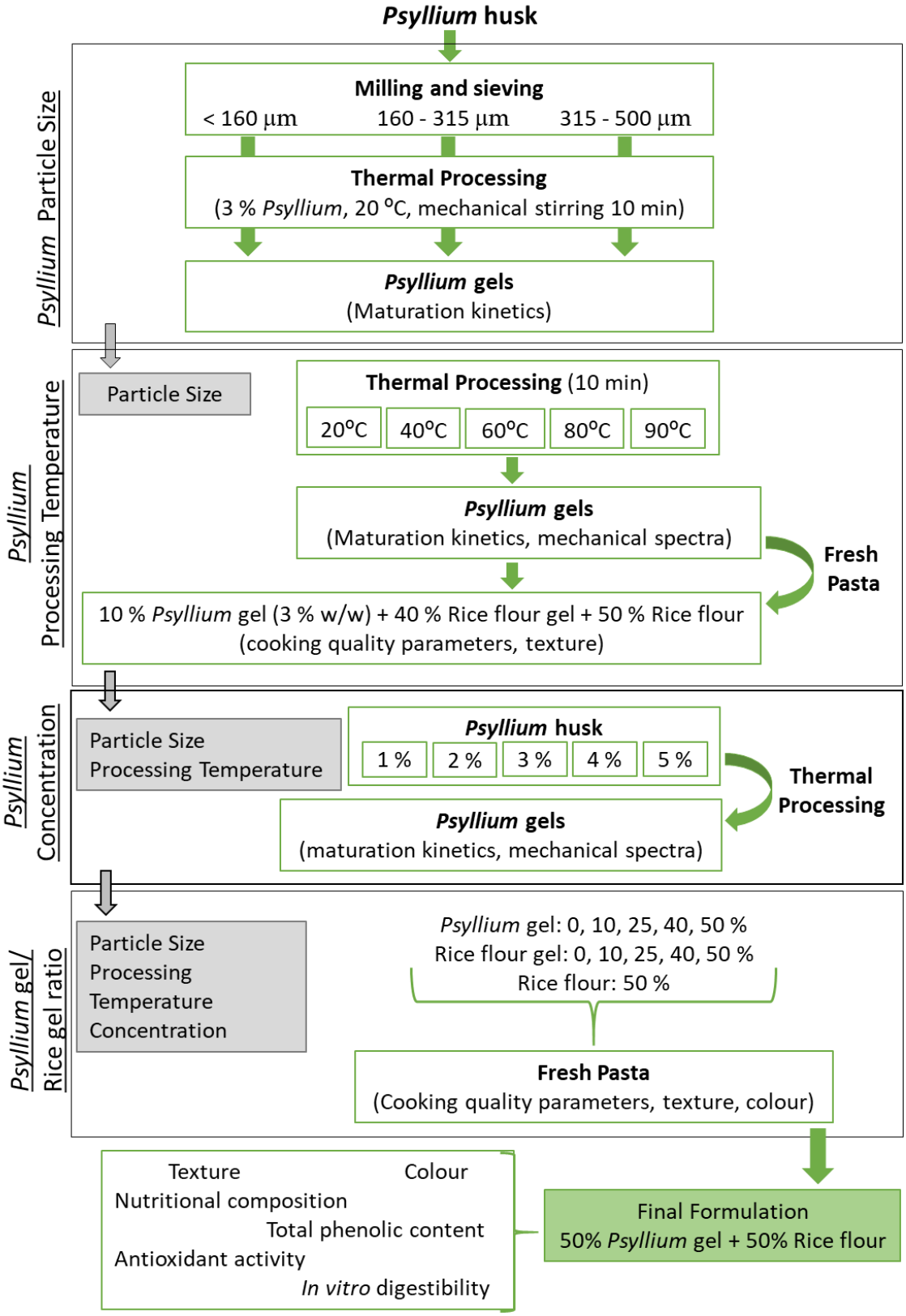

Figure C. Schematic procedure of chapter 3.

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Psyllium husk gel to reinforce structure of gluten-free pasta?

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ABSTRACT

Gluten-free pasta is a technological challenge. The effect of *Psyllium* particle size, processing temperature and gel concentration on the quality of rice-based pasta was investigated. The rheological properties, i.e. maturation kinetics and mechanical spectra, of the *Psyllium* gels were studied and optimal conditions were set: 160–315 μm particle size, 4 g/100 g *Psyllium* husk concentration thermally processed at 40 °C. Cooking quality parameters, texture properties, nutritional composition, antioxidants and digestibility of pasta were determined. Consequently, the use of *Psyllium* husk in gluten-free pasta showed good overall properties. Moreover, the pre-gelatinization step of rice flour can be eliminated, resulting in a final gluten-free pasta formulation with *Psyllium* gel and rice flour (50/50) with high digestibility.

1. Introduction

In the last decade, the worldwide gluten-free (GF) food market increased 83%, with pasta products having an estimated annual growth rate of 12.3% until 2022 (Chauvin, 2019). Despite this massive increase, GF food products are still not very sensory appealing, are nutritionally unbalanced, often show texture problems, and its price is at least 75% higher than its gluten similar (Singh & Whelan, 2011; Vici, Belli, Biondi, & Polzonetti, 2016).

The main issue in GF pasta production is the lack of gluten, the structuring element of traditional *durum* wheat pasta. In the absence of this protein matrix, which promotes pasta cohesiveness, viscosity, extensibility and elasticity (Lazaridou, Duta, Papageorgiou, Belc & Biliaderiset, 2007), it is necessary to consider other *structure builders*. Previous strategies were focused on thermal and high pressure treatments to build up structure of GF flours (Jalali, Sheikholeslami, Elhamirad, Khodaparast, & Karimi, 2020; Vallons, Ryan, & Arendt, 2011); the use of pulses and pseudo-cereals (Burešová et al., 2017); hydrocolloids (e.g. hydroxypropyl methylcellulose (HPMC), xanthan gum, locust bean gum) and proteins (e.g. transglutaminase, casein, albumin) (Crockett, Ie, & Vodovotz, 2011; Storck et al., 2013).

A gluten free pasta formulation, based on pre-gelatinized rice flour from broken grains (a by-product of the rice industry), was previously developed (Fradinho, Sousa, & Raymundo, 2019a). However, this pasta had not enough mechanical resistance compared to wheat-based fresh pasta. A way to tackle this is fibre enrichment, which generally contributes positively to the preservation of the microstructure of pasta and entraps starch granules, thereby improving the dough and cooking properties (Mercier, Moresoli, Mondor, Villeneuve, & Marcos, 2016).

Following consumer trends, both researchers and the food industry are focused on the development of healthy food products with clean labels (Angus & Westbrook, 2019) to fulfil consumer expectations and needs for more natural foods, made from ingredients that are recognized, sustainable, locally produced and authentic (Asioli et al., 2017). In this context, replacing synthetic (e.g. hydroxypropyl methylcellulose, HPMC) and natural hydrocolloids (e.g. xanthan gum) with alternative sources of biopolymers (flaxseed, chia, *Psyllium* husk) could be a valid technological approach to improve both texture and nutritional properties of foods.

The plants of *Plantago* genus, the source of *Psyllium* husk, have been used worldwide in traditional medicine (Samuelsen, 2000), but only in 2012 FDA recognized the positive effect of *Psyllium'* soluble fibre on coronary heart disease risk reduction (FDA, 2012). The structure of *Psyllium* husk arabinoxylan is also able to resist gut fermentation (Pollet et al., 2012), acting as a prebiotic agent with health effects, i.e. i) increases in bifidobacteria and lactobacilli, ii) production of beneficial metabolites, iii) increases in calcium absorption, iv) decreases in protein fermentation, v) decreases in pathogenic bacteria populations, vi) decreases in allergy risk, vii) effects on gut barrier permeability, and viii) improved immune system defence (Broekaert et al., 2011; Carlson,

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Erickson, Lloyd, & Slavin, 2018). *Psyllium* husk has multifunctional applications in several industrial fields due to its unique gelling properties (Haque, Richardson, & Morris, 1993), low cost, biodegradability and eco-friendliness (Belorio, Sahagún, & Gómez, 2019; Thakur & Thakur, 2014).

Furthermore, several authors focus on the use of *Psyllium* as a structure builder that mimics the gluten matrix in bread (Ziemichód, Wójcik & Różyło, 2019).

This study aims to determine the optimal processing conditions for *Psyllium* husk, suitable for pasta dough incorporation, to build-up the structure and to enhance the rheology and nutritional properties and *in vitro* digestibility of the GF pasta, compared to a commercial reference.

2. Materials and methods

2.1. Materials

Psyllium husk (lot 047,058-02, Solgar, USA) from India and rice flour (lot 3411/18, Ceifeira, Dacsa Atlantic, Portugal) came from the local market. A dried commercial rice spaghetti (lot L1223, Urtekram, Denmark) was used for comparison.

2.2. Experimental design

Gluten free pasta formulation optimized in a previous work (Fradinho et al., 2019a) with rice flour pre-gelatinized gel and rice flour (50/50) was used as control. Preliminary trials were performed to assess the range of *Psyllium* husk concentration (1–5 g/100 g). The following conditions were considered: i) *Psyllium* particle size - *psyllium* husk milled by centrifuge mill (Pulverisette 14 Premium, Fritsch, Idar-Oberstein, Germany) at 6000 rpm for 300 s and sieved. Three particle fractions (<160 μm; 160–315 μm; 315–500 μm) separated, and the distribution of particle size established. Gels were prepared adding 3 g/100 g (d.b.) *Psyllium* husk to distilled water at 20 °C under mechanical stirring for 10 min, covered with aluminium foil to prevent evaporation. Rheology studies were performed according to the procedure described in section 2.3. The best *Psyllium* particle size was determined based on the fastest maturation gel, a technological advantage, and taking into account the amount of each particle size fraction obtained after milling and sieving; ii) Processing temperature - gels of 3 g/100 g were prepared at 20 °C, 40 °C, 60 °C, 80 °C and 90 °C under mechanical stirring for 10 min. Rheology studies were performed to exclude gels with the lowest maturation kinetics. Subsequently, pasta was prepared according to the previously studied conditions reported by Fradinho et al. (2019a), replacing a fraction of the rice flour gel with the matured *Psyllium* gel, resulting in a 10/40/50 formulation. Pasta samples were characterized in terms of cooking quality parameters and texture. The next selection step was concentration: iii) *Psyllium* concentration - *psyllium* gels were prepared with 1, 2, 3, 4 and 5 g/100 g (d.b.) at particle size and temperature conditions chosen considering the best results obtained in the previous trials. Rheology studies were performed to exclude the gels with the lowest maturation kinetics. Next followed the fine tuning of the *Psyllium* gel/Rice gel ratio: iv) *Psyllium* gel/Rice gel ratio - pasta formulations were prepared by combining *Psyllium* gel and rice flour gel at 10/40, 25/25, 40/10 and 50/0 ratios, added to the other 50% of rice flour. Pasta developed was analysed for cooking quality parameters and texture properties.

2.3. Psyllium gel rheology measurements

Rheology behaviour of *Psyllium* gels was assessed by small-amplitude oscillatory shear (SAOS) measurements in a controlled stress rheometer (MARS III Haake, Thermo Scientific, Karlsruhe, Germany) with temperature control by an UTC-Peltier system. Parallel plate geometry (35 mm diameter) and 1.5 mm gap rough plates, to avoid slippage. Exposed edges of samples were covered with paraffin oil to prevent evaporation. Prepared *Psyllium* gels were immediately poured into the bottom plate of the rheometer to perform all the measurements.

Time sweeps were performed at 20 °C and 1 Hz for 180 min to obtain the maturation kinetics of the gels. For fully matured gels within the period mentioned, frequency sweep tests were performed from 0.01 to 100 Hz, at 20 °C, with a stress within the linear viscoelasticity region, previously determined by a stress sweep at 1 Hz. The storage modulus (G') and loss modulus (G'') were the highlighted parameters. Each test was run in triplicate.

2.4. Fresh pasta preparation and sampling

All pasta formulations (200 g) were prepared in triplicate, by mixing the ingredients in a food processor (Bimby TM31, Vorwerk, Wuppertal, Germany) for 3 min (speed 4) at 25 °C. Then, the dough was sheeted and laminated as "tagliatelle" using a benchtop pasta machine (Atlas 150 Wellness, Marcato, Italy) and these strands were covered by aluminium foil and allowed to equilibrate for 15 min at 25 °C.

For biochemical analysis, antioxidant activity and *in vitro* digestibility determinations pasta samples were cooked for 1 min, frozen, lyophilized (Scanvac Coolsafe 55-4, Labogene, Allerød, Denmark), crushed into powder (<0.5 mm) and stored in a desiccator at room temperature. The assessment of the cooking quality parameters and texture properties of pasta were performed within 2 h of pasta preparation.

2.5. Pasta analysis

2.5.1. Cooking quality evaluation of pasta

Cooking time of the control pasta (50 gelatinized rice flour/50 rice flour) was assessed in the authors previous study (Fradinho et al., 2019a). This pasta shape (tagliatelle) is very thin and partly composed of gelatinized starch, so 1 min is enough to cook it, without breaking the pasta strands. Replacing gelatinized starch with Psyllium gel did not change the cooking time.

Cooking quality parameters: water absorption (WA), swelling power (SP) and cooking loss (CL), were determined as earlier reported by Fradinho et al. (2019a). At least three measurements were performed.

2.5.2. Texture analysis

Cooked pasta texture parameters were determined using a texturometer TA.XTplus (Stable MicroSystems, Godalming, UK) with a 5 kg load cell and a blade set with guillotine (HDP/BSG) and a Kieffer Dough & Gluten Extensibility Rig (A/KIE), in a 20 °C controlled temperature room. Pasta samples were cooked in boiling water for 1 min, rinsed with distilled water and drained. Cutting and extensibility tests were performed within 15 min after draining, according to the procedure earlier described in Fradinho et al. (2020). Each test was replicated eight times.

2.5.3. Proximate composition and antioxidant capacity determination of cooked pasta

Crude protein, total lipids and total carbohydrate contents were determined following Lowry, Rosebrough, Farr, and Randall (1951), Marsh and Weinstein (1966) and Dubois, Gilles, Hamilton, Rebers, and Smith (1956), respectively. Moisture and ash were analysed (ISTISAN Report 1996/34, method B and ISTISAN Report 1996/34). The total phenolic content (TPC) was determined using the Folin Ciocalteu assay according to Ganesan, Kumar, and Bhaskar (2008).

To evaluate the radical scavenging capacity of the cooked pasta samples, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Rajauria, Jaiswal, Abu-Ghannam, & Gupta, 2013) was performed. The antioxidant capacity of the samples was expressed in terms of μg of Vitamin C Equivalent Antioxidant Capacity (VCEAC) per gram of sample (ascorbic acid calibration curve: 0–10 mg mL^{-1}, $R^2 = 0.992$) and corresponding Radical Scavenging Activity (RSA) (%). Two blank assays, one without samples and another without reagents, were also

performed. Analyses were repeated in triplicate and performed in cooked pasta samples, previously lyophilized.

2.5.4. In vitro digestibility tests

The *in vitro* digestibility (IVD) of cooked pasta samples was assessed according to the Boisen & Fernández method (1997) modified by Niccolai, Zittelli, Rodolfi, Biondi, and Tredici (2019). Briefly, 1 g of lyophilized sample was weighed (particle size ≤ 1 mm) and transferred to 250 mL conical flasks. Then, 25 mL of phosphate buffer (0.1 M, pH 6.0) was added and mixed, followed by 10 mL of 0.2 M HCl and pH was adjusted to 2.0. A freshly prepared pepsin water solution (3 mL) containing 30 mg of porcine pepsin (0.8 FIP-U/mg) was added, and the flasks were incubated at 39 °C for 6 h with constant agitation (150 rpm). Subsequently, phosphate buffer (10 mL, 0.2 M, pH 6.8) and NaOH solution (5 mL, 0.6 M) were added to each sample and pH was adjusted to 6.8. A freshly prepared pancreatin ethanol:water solution (10 mL, 50:50 v/v) containing 500 mg of porcine pancreatin (42,362 FIP-U/g) was added to each sample and the flasks were incubated at 39 °C, 150 rpm, for 18 h. A reagent blank without sample was also prepared. The undigested residues were collected by centrifugation at 18,000×*g* for 30 min and washed with deionised water. This procedure was repeated twice, and the final supernatant was filtered on glass-fibre membranes (47 mm Ø, pore 1.2 μm). The pellet and membranes were dried at 80 °C for 6 h, and then at 45 °C until constant weight. The dry matter, crude protein, and carbohydrate *in vitro* digestibility (%) of all pasta samples was calculated from the difference between the initial biomass and the undigested dry matter, crude protein, and carbohydrate biomass (after correction for the blank assay), expressed as percentage of the initial dry matter, crude protein, and carbohydrate biomass. Casein (Sigma Aldrich Corp., St. Louis, USA) was used as the reference material for 100% digestibility.

2.6. Statistical analysis

Experimental data is presented as average ± standard deviation (s.d.). Significant differences between samples were assessed by one-way ANOVA followed by Tukey's HSD test at 95% confidence level ($p < 0.05$) using RStudio (version 1.1.463 – © 2009–2018 RStudio, Inc.).

3. Results and discussion

3.1. Psyllium gel settings

3.1.1. Psyllium husk particle size

After milling and sieving, *Psyllium* husk showed the following particle size distribution: 12.1% with <160 μm, 38.4% with 160–315 μm and 49.1% with 315–500 μm.

The maturation kinetic curves of *Psyllium* gels, prepared at 20 °C with different particle sizes, were monitored through the evolution of G′ and G″ with time (Fig. 1). As observed, particle size impacts the rheology of *Psyllium* gel. This mucilage has a high water uptake, dependent upon a multitude of factors, such as particle size, type of milling and processing temperature (Raymundo, Fradinho, & Nunes, 2014; Van Craeyveld, Delcour, & Courtin, 2008), which explains the different gel profiles.

The steady value of G′, when the gel reaches a stable and fully developed structure, can be defined as the G'_{eq}, i.e. the value of G′ at the pseudo-equilibrium-state at infinite time (Nunes, Batista, Raymundo, Alves, & Sousa, 2003).

$$G'_{eq} = \lim_{t \to \infty} G'(t) \tag{2}$$

or alternatively

$$G'_{eq} = \lim_{1/t \to 0} G'(t) \tag{3}$$

The experimental data can be fitted to the following second order exponential decay equation:

$$G'(k) = y_0 + A_1 e^{-k/b1} + A_2 e^{-k/b2} \tag{4}$$

where y_0, A_1, A_2, b_1 and b_2 are the equation parameters and k is the reciprocal time, i.e. 1/t. Table 1 presents G'_{eq} values extrapolated along with the parameters of Eq. (4), G′ obtained at 180 min and the maturation index, given by the ratio (G'_{180min}/G'_{eq}) x 100 (Batista et al., 2012).

The shorter maturation time of 5 h was obtained for *Psyllium* gel with 160–315 μm particle size, against almost 7 h and 8.5 h of 315–500 μm and <160 μm, respectively. This range is close to a coarse flour (132–200 μm), recommended by de la Hera, Rosell, and Gomez (2014) and Gómez and Martínez (2016) in terms of bread quality and *in vitro* starch digestibility.

After milling, the intermediate particle size fraction had about 3 times more quantity than the lower particle size fraction. Also, there was

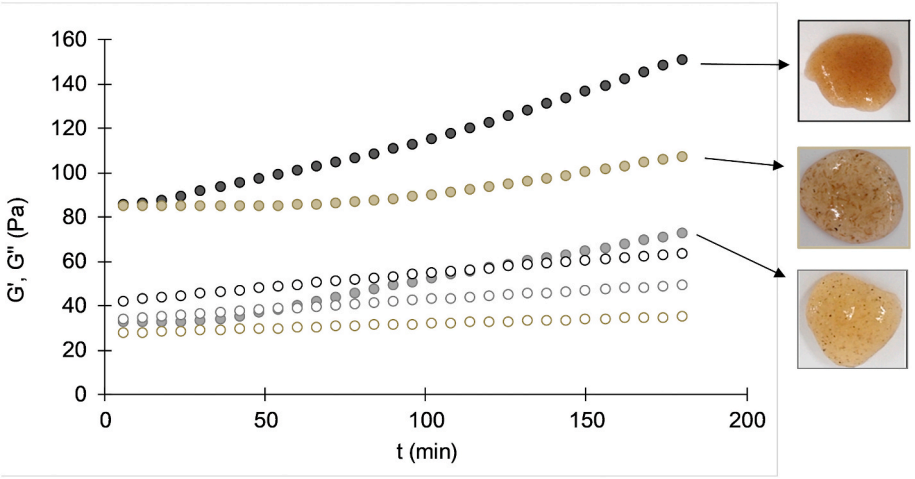

Fig. 1. Maturation kinetic curves of *Psyllium* gels with <160 μm (●), 160–315 μm (●) and 315–500 μm (●) particle size. Close symbol (G′), open symbol (G″).

Table 1
Parameters of exponential decay and calculated G'_{eq} and $G'_{180\ min}/G'_{eq}$ x 100 of *Psyllium* gels with different particle sizes.

	y_0 (Pa)	A_1 (Pa)	b_1 (1/min)	A_2 (Pa)	b_2 (1/min)	G'_{eq} (Pa)	$G'_{180\ min}$ (Pa)	$G'_{180\ min}/G'_{eq}$ x 100 (%)
Particle size								
<160 μm	85.7	37.7	0.0179	302.0	0.0027	425.4	151.0	35.5
160–315 μm	33.0	46.1	0.0063	46.1	0.0063	125.2	72.8	58.1
315–500 μm	85.1	79.3	0.0028	79.3	0.0028	243.7	107.3	44.0

Reduced Chi-square $\chi^2 = 1.7–3.0$; $R^2 = 0.997–1.000$.

the possibility of further milling the 315–500 μm fraction in order to increase the amount of 160–315 μm *Psyllium* fraction. For these reasons, the range of 160–315 μm particle size was selected.

3.1.2. *Psyllium processing temperature*

Based on previous work by Haque et al. (1993), a range of temperatures between 20 and 90 °C was selected for *Psyllium* gel processing. Gels were subjected to isothermal time sweep measurements (Fig. 2a) followed by a frequency sweep at 20 °C (Fig. 2b). Except for the *Psyllium* gel processed at 20 °C, all the other *Psyllium* gels attained full maturation almost immediately (Fig. 2a). The mechanical spectra of gels were all similar, with G′ higher than G″ with some frequency dependence over the 0.01–10 Hz frequency range studied, typical of a weak gel-like structure, where molecular associations tolerate low-amplitude oscillation but are broken down under steady shear, giving rise to flow (Fig. 2b). An increasing G' with increasing processing temperature is also observed, which reflects the temperature dependence of *Psyllium* gels, already described by Haque et al. (1993). As expected, at 20 °C the gel structure is weaker and more frequency dependent, therefore gels processed at 40 °C, 60 °C, 80 °C and 90 °C were used to produce fresh

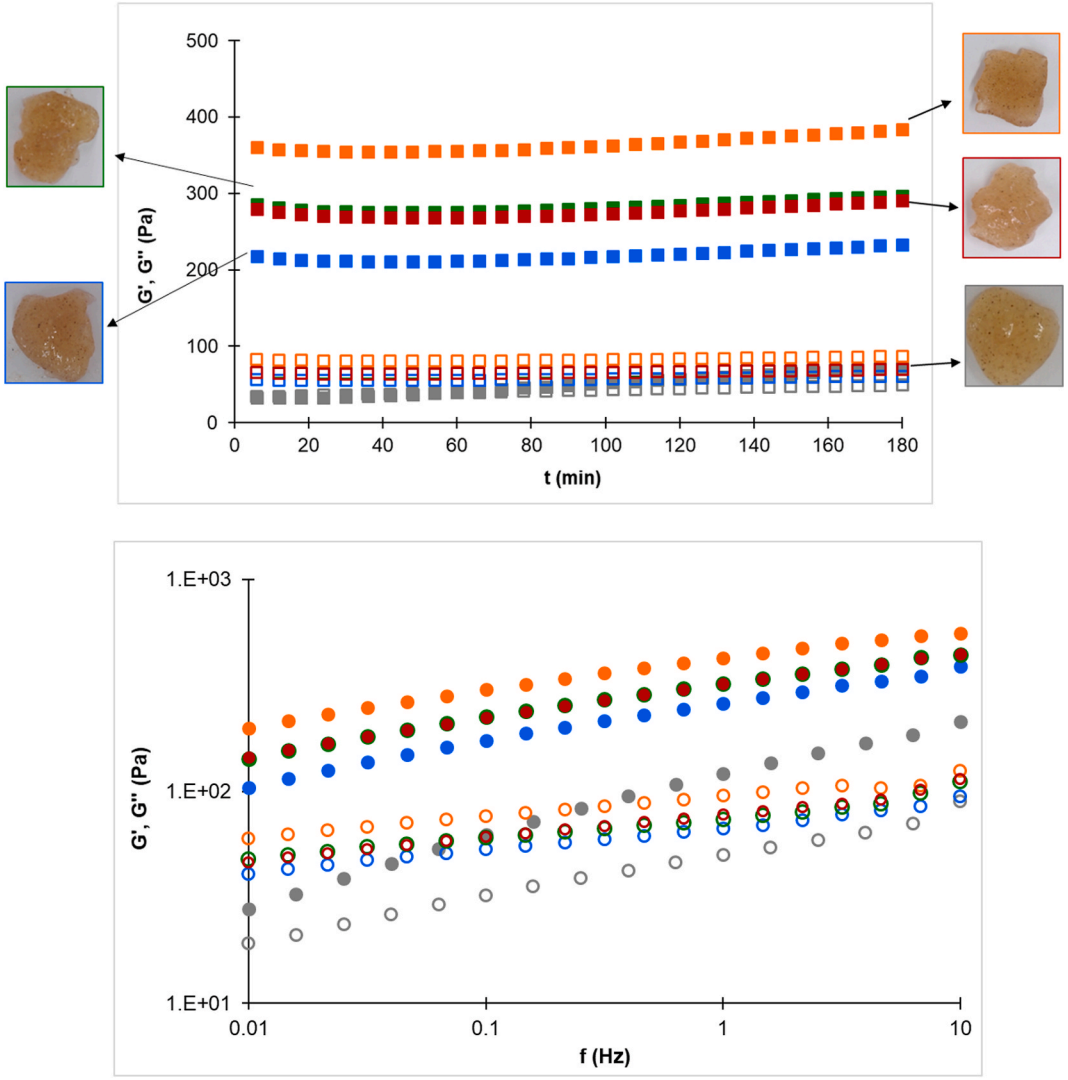

Fig. 2. Maturation kinetic curves (a) and mechanical spectra (b) of 3 g/100 g *Psyllium* gels thermally processed at 20 °C (■), 40 °C (■), 60 °C (■), 80 °C (■) and 90 °C (■). Close symbol (G′), open symbol (G″).

pasta, to assess the best processing temperature for pasta incorporation.

Based on a gluten-free fresh pasta developed in a previous work (Fradinho et al., 2019a), a fraction of the rice flour gel was replaced by *Psyllium* gel, resulting in a final formulation composed by 10% *Psyllium* gel, 40% rice flour gel and 50% rice flour. All pastas presented similar water absorption (WA) and cooking loss (CL) values and comparable to the Control pasta without *Psyllium* gel (WA: 43.5–49.0 g/100 g; CL: 1.1–1.7 g/100 g). However, swelling power (SP) of *Psyllium* pastas (except 90 °C) was higher (SP: 0.85–0.92 mL/g) than of the Control (SP: 0.79 mL/g). For the texture parameters, all *Psyllium* pasta samples showed significant ($p < 0.05$) lower firmness values (1.94–2.04 N) than the Control (2.1 N). This could be related to *Psyllium* husk hydration properties that increased the water imprisoned into the pasta matrix, as observed in swelling values. Adhesiveness is a negative feature in pasta, lower at 40 °C ($A_{40°C}$ = 0.025 N; $A_{Control}$ = 0.109 N), maintaining a firmness value close to the Control. Although *Psyllium* gel processed at 90 °C also led to pasta with similar texture characteristics, a higher processing temperature means a higher energy input, which translates in higher processing costs. For this reason, the processing temperature of 40 °C was selected.

3.1.3. *Psyllium husk concentration*

The maturation kinetic curves of *Psyllium* gels processed at 40 °C with concentrations between 1 and 5 g/100 g were conclusive for full maturation within the time considered (180 min) and were subsequently characterized in terms of their mechanical spectra (Fig. 3).

Besides particle size and processing temperature dependence, *Psyllium* gels, as expected, also show concentration dependence with two

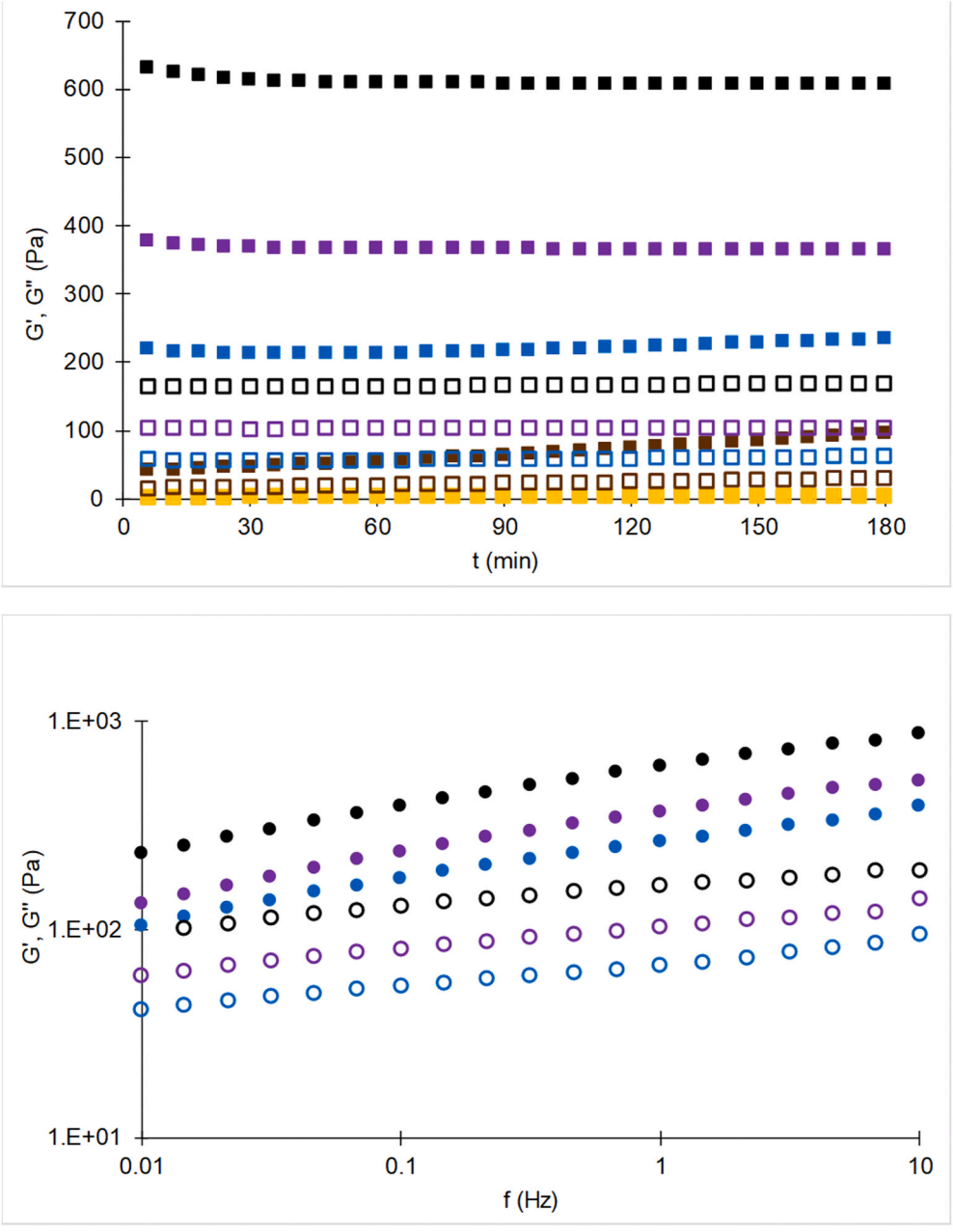

Fig. 3. Maturation kinetic curves (a) and mechanical spectra (b) of gels prepared with 1 (■), 2 (■), 3 (■), 4 (■) and 5 g/100 g (■) *Psyllium* husk. Close symbol (G′), open symbol (G″).

groups of spectra: 1 and 2 g/100 g (Fig. 3a) and 3, 4 and 5 g/100 g curves (Fig. 3b). At 1 g/100 g the behaviour is similar to a suspension since G″ is close to G'. Although 2 g/100 g showed to be more structured with G′ over G″ with time, both 1 and 2 g/100 g systems were not fully maturated within 180 min. For the second group of *Psyllium* concentrations the spectra are from a similar structure, as loss tangent is not affected by concentration (tan $\delta_{1\ Hz}$ = 0.256–0.278), typical of a weak gel-like behaviour (Fig. 3b).

Based on the previous rheology measurements, there is no obvious reason for choosing one concentration over another, in terms of pasta production. All three *Psyllium* concentrations were used in the next trials.

3.1.4. *Psyllium gel/rice flour gel ratio*

Different *Psyllium* gel and rice flour gel ratios were tested: 0/50, 10/40, 25/25, 40/10 and 50/0 and respective formulations of rice pasta were prepared using the conditions selected in the previous trials, i.e., 50% rice flour (Fradinho et al., 2019a) and *Psyllium* husk (3–5 g/100 g) with 160–315 μm particle size thermally processed at 40 °C.

As observed in Fig. 4, the formulations within the grey backgrounds produced pastas with high stickiness or with evident breaking points emerged during lamination. The final step of pasta development was performed considering the other formulations (blue shadowed, in Fig. 4). All resulting pasta samples were characterised for cooking quality (Fig. 5) and texture (Fig. 6).

Pasta cooking behaviour is a critical step for its quality perception by the consumers. The replacement of gelatinized rice flour by *Psyllium* gel did not affect significantly ($p < 0.05$) the pasta hydration capacity in terms of swelling and water absorption (Fig. 5). However, *Psyllium*' addition had a significant ($p < 0.001$) positive effect on the decrease of leached solids into the cooking water (cooking loss), especially at the highest concentrations (4 and 5 g/100 g). Some works on gluten pasta with fibre addition report contrasting results, i.e. fibre addition increased the cooking loss. They relate this behaviour to the competitive hydration tendencies of the fibres, weakening the gluten network, which is responsible for retaining the solids during cooking (Tudorică, Kuri, & Brennan, 2002). In fact, Foschia, Peressini, Sensidoni, Brennan, and Brennan (2015) found more than 10 g/100 g cooking loss in semolina pasta with *Psyllium* husk. In the present study, due to the absence of gluten, the pasta network was mainly formed by gelatinized starch. Adding *Psyllium* in gel form, and not in powder, most likely decrease *Psyllium*' hydration competitiveness, showing a complementarity with the starch gel to build up the GF pasta internal matrix, hindering the leaching of materials into the cooking water. Gasparre and Rosell (2019) results seem to support this hypothesis referring that hydrocolloid addition (xanthan gum, inulin and carboxymethyl cellulose) to GFpasta significantly decreased the cooking loss, but still showing much higher values (13.7–16.5 g/100 g) compared to (0.6–1.5 g/100 g) of the present work.

Texture results (Fig. 6) show that the firmness and adhesiveness of GF pastas depend on the *Psyllium* concentration as well as on the ratio of gelatinized starch to *Psyllium* gel. As earlier reported (Bustos, Perez, & León, 2013), fibre-enriched pasta has low firmness, and high adhesiveness due to amylose leaching to the cooking water. However, in this study, all *Psyllium* incorporated pastas were less adhesive than the Control, probably related to the gelling properties of this material,

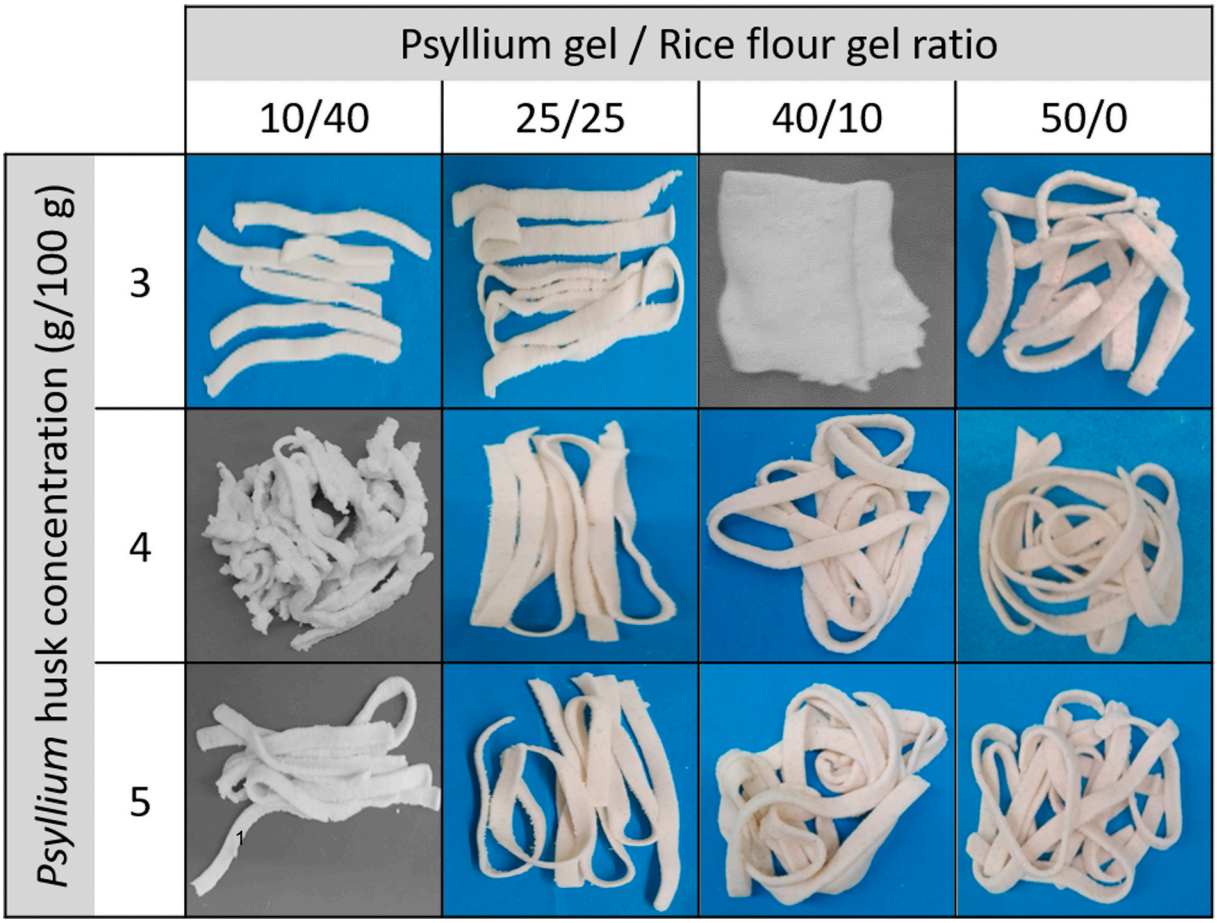

Fig. 4. Pasta dough formulations produced with 3–5 g/100 g *Psyllium* husk and different *Psyllium*. gel/rice gel ratios (10/40, 25/25, 40/10 and 50/0). The pastas with blue background were selected for further analyses. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

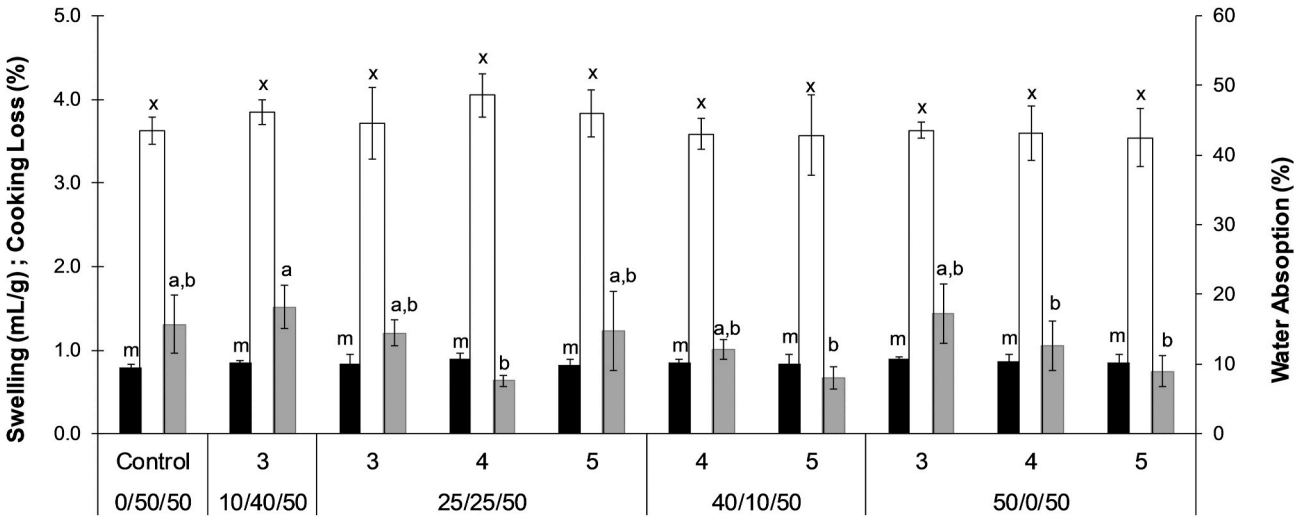

Fig. 5. Cooking quality parameters (swelling - ■; water absorption - □; cooking loss - ■) of pasta formulations produced with 3–5 g/100 g *Psyllium* husk and different *Psyllium* gel/rice gel ratios (10/40, 25/25, 40/10 and 50/0), and the control (without *Psyllium* gel). Data shown is mean ± SD, n = 4. Different letters in the same parameter show significant differences ($p < 0.001$, one-way ANOVA post-hoc Tukey test).

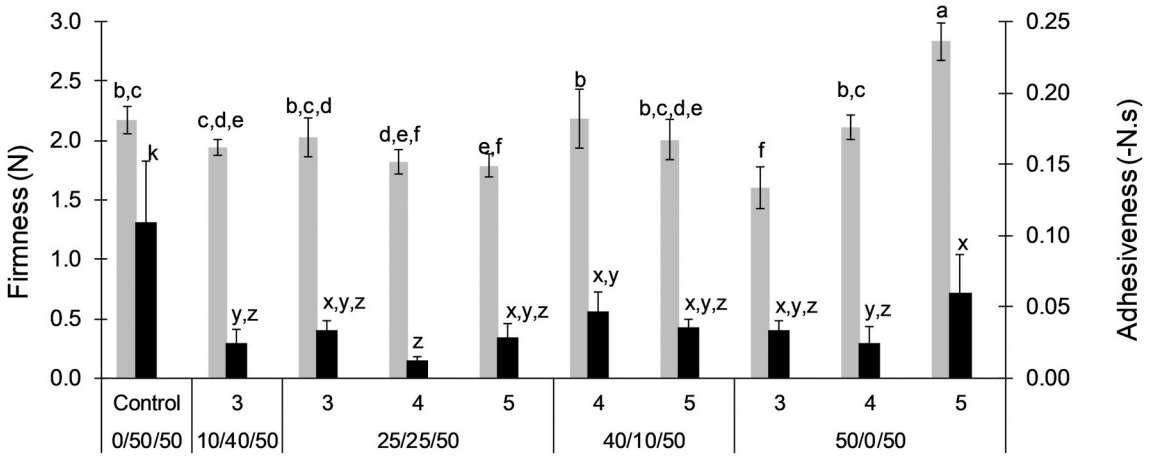

Fig. 6. Firmness (■) and adhesiveness (■) of pasta formulations produced with 3–5 g/100 g *Psyllium* husk and different *Psyllium* gel/rice gel ratios (10/40, 25/25, 40/10 and 50/0), and the control (without *Psyllium* gel). Data shown is mean ± SD, n = 6. Different letters in the same parameter show significant differences ($p < 0.001$, one-way ANOVA post-hoc Tukey test).

promoting a more cohesive structure with lower cooking loss. Similarly, Belorio et al. (2019) stated that the incorporation of pre-hydrated *Psyllium* was responsible for a more cohesive dough, contributing to reduce the oil percentage in cake formulations.

Regarding firmness results, for the same gelatinized rice flour content (e.g. 25/25/50) there seems to be a tendency for firmness decrease for the formulations with increasing Psyllium concentration. On the other hand, for formulations without gelatinized rice flour (50/0/50), increasing *Psyllium* gel concentration resulted in higher pasta firmness after cooking. This could be due to a sort of competitive phenomena between the two gels, hindering the development of the full potential of Psyllium to build up the internal structure. Likewise, Gasparre and Rosell (2019) described a rise of firmness along with a significant reduction of adhesiveness of GF pasta in the presence of hydrocolloids.

It is noteworthy that only pasta samples with 4 g/100 g *Psyllium* (25/25/50, 40/10/50, 50/0/50) allowed the performance of extensibility measurements, with Resistance to extension (R_{max}) ranging from 0.58 to 0.64 N and distance until rupture (ER_{max}) from 5.23 to 5.94 mm. Although these values were lower than the ones obtained for wheat pasta (Fradinho et al., 2020) they show a positive result for GF pastas.

Based on these results, the GF pasta composed of only rice flour (without rice gel) and *Psyllium* gel (4 g/100 g) at a 50/50 ratio was selected, which eliminates the rice flour pre-gelatinization step, making industrial processing far easier. In addition, starch gelatinization increases glycemic index (GI) of the food matrices (Parada & Aguilera, 2011), so replacing this material with *Psyllium* husk, which is a fibre, is a promising alternative to develop GF pasta with health benefits and lower GI.

3.2. Proximate composition, antioxidant capacity and in vitro digestibility

In Table 2 the proximate composition, the antioxidant capacity and the *in vitro* digestibility of the optimized cooked Psyllium pasta formulation (PP) against the control are shown. A commercial rice pasta (CRP) and a wheat pasta (WP) were also characterized for comparison.

In terms of proximate composition, the PP pasta showed very low lipid content, consistent with the low lipid content of the raw materials, namely *Psyllium* husk and rice flour (Fradinho et al., 2019a; Raymundo et al., 2014), value in line with WP and significantly ($p < 0.05$) lower than the commercial rice pasta (CRP).

Although PP showed a higher carbohydrate content than the other pastas, this can be attributed to the higher fibre content of *Psyllium*

Table 2
Proximate composition, antioxidant capacity and *in vitro* digestibility of cooked pasta samples with gelatinized rice flour (Control), *Psyllium* (PP), commercial rice pasta (CRP) and wheat pasta (WP).

Samples	Biochemical composition					Antioxidant capacity		*In vitro* digestibility		
	Protein	Lipids	Ash	Carbohydrates	TPC	RSA	VCEAC	Dry matter	Protein	Carbohydrate
	(g/100 g, dry basis)				(mg GAE/g)	(%)	(μg/g)	(%)		
Control	3.6 ± 0.3^{b}	1.4 ± 0.1^{b}	0.7 ± 0.0^{b}	83.8 ± 5.2^{b}	$0.22 \pm 0.03^{b,c}$	$52.05 \pm 3.92^{b,c}$	0.55 ± 0.05^{a}	97.61 ± 0.31^{a}	32.06 ± 4.68^{b}	97.45 ± 0.26^{a}
PP	3.9 ± 1.0^{b}	1.3 ± 0.1^{b}	0.4 ± 0.4^{b}	93.2 ± 4.1^{a}	0.09 ± 0.05^{c}	50.92 ± 2.02^{c}	0.52 ± 0.02^{a}	92.95 ± 1.19^{b}	36.33 ± 3.07^{b}	93.99 ± 0.26^{b}
CRP	4.3 ± 1.4^{b}	2.3 ± 0.2^{a}	1.4 ± 0.1^{a}	79.2 ± 3.1^{b}	0.72 ± 0.10^{a}	$55.72 \pm 5.53^{a,b}$	0.59 ± 0.08^{a}	91.73 ± 0.23^{b}	40.29 ± 1.48^{b}	94.82 ± 1.10^{b}
WP*	6.0 ± 1.2^{a}	1.4 ± 0.1^{b}	0.8 ± 0.1^{b}	83.1 ± 2.7^{b}	0.35 ± 0.18^{b}	46.62 ± 3.19^{d}	0.44 ± 0.03^{b}	94.52 ± 2.10^{b}	62.61 ± 8.98^{a}	93.84 ± 0.51^{b}

Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test). * Fradinho et al. (2020).

(Raymundo et al., 2014), rendering a pasta with around 6 g/100 g (d.b.) total fibre content, as the authors already stated in a previous work (Fradinho, Raymundo, Sousa, Domínguez, & Torres, 2019b). Regarding the antioxidant activity, the results revealed that all GF pastas showed significantly ($p < 0.05$) higher antioxidant activity than wheat pasta. *Psyllium* incorporation did not affect the antioxidant activity (RSA and VCEAC) of GF pasta, neither its total phenolic content.

The *in vitro* digestibility (IVD) of the cooked pasta samples was determined by an enzymatic method using pepsin and pancreatin. Due to the well-recognized influence of fibre on starch digestion, preventing excess glucose absorption, the addition of dietary fibre to cereal-based foods has been investigated as an alternative to lower its GI (Bustos et al., 2013; Oh, Bae, & Lee, 2014). To our knowledge, the *in vitro* digestion-retarding effect of *Psyllium* husk in rice-based foods has not been examined. GF food products generally have higher GI that their wheat counterparts (Foster-Powell, Holt, & Brand-Miller, 2002; Berti, Riso, Monti, & Porrini, 2004), which is also confirmed by the present work, when comparing WP and Control IVD values. This is due to the raw materials used in GF food production (e.g. rice, corn) which have high starch digestion rates (Toutounji et al., 2019).

As observed (Table 2), *Psyllium* addition contributed significantly ($p < 0.05$) to the decrease of dry matter and carbohydrate digestibility in PP pasta when compared to the Control. In a previous work (Koh, Kasapis, Lim, & Woo, 2009), found that the *in vitro* digestion of rice-based noodles was retarded by the alginate addition. According to Parada, Aguilera, and Brennan (2011), due to the hygroscopic nature of dietary fibres, they reduce water available for starch gelatinization, consequently reducing starch digestibility. Although similar carbohydrate IVD results were obtained for PP and WP pastas, the structure of *Psyllium* husk arabinoxylan is able to withstand fermentation in the gut (Pollet et al., 2012), acting as prebiotic (Broekaert et al., 2011). *Psyllum* husk contains a high amount of arabinoxylan (Fischer et al., 2004). Arabinoxylan of *Psyllum* is highly branched non-starch polysaccharide with a main chain of densely substituted β-(1,4) linked xylopyranose residues. Single arabinofuranose and xylopyranose residues, or short side chains consisting of these monosaccharides, are attached at positions 2 and/or 3 of the main chain xylopyranose residues (Fischer et al., 2004). Arabinoxylan oligosaccharides selectively stimulate the growth and activity of beneficial colon bacteria. Bifidogenic effects in the gut include the growth of health-promoting bacteria (such as lactobacilli and bifidobacteria), the increase in production of short-chain fatty acids (such as butyric and propionic acid) which are believed to be positive for colonic health, and the decrease of toxic bacterial metabolites (such as polyamines and ammonia) (Broekaert et al., 2011).

Protein digestibility values of PP were similar to the ones of CRP and Control, i.e, all the GF samples showed similar protein digestibilities. The average protein digestibility value of GF pastas analysed in this work (36.2%) is comparable to that of pastas made with *durum* wheat semolina + gluten powder, *durum* wheat semolina dried at different temperatures, and corn (39.2, on average 38.4%, and 34%, respectively) (Laleg, Barron, Santé-Lhoutellier, Walrand, & Micard, 2016; Palavecino, Ribotta, León, & Bustos, 2019; Petitot, Abecassis, & Micard, 2009). Interestingly, Laleg et al. (2016) reported a similar value of protein digestibility for wheat pasta (42%) compared to one found in the present work for CRP (40%), highlighting like GF pastas did not present alteration in protein digestibility compared to conventional wheat-based pastas. A significantly lower protein digestibility between Control and CRP pastas developed in this study and rice-based pastas developed in different studies available in the literature has been found (Obulesu & Bhagya, 2006; Rafiq, Sharma, & Singh, 2017). It is worth pointing out that differences in pH, mineral type, ionic strength and digestion time, which alter enzyme activity and other phenomena, may also considerably alter digestibility results in the different studies (Minekus et al., 2014). To fully clarify these points, further studies aimed at evaluating the structure of GF pastas protein after *in vitro* enzymatic digestion compared to commercial pastas are necessary. However, the WP with higher protein content, also showed higher protein digestibility, consistent with findings from other works (e.g. Susanna & Prabhasankar, 2013).

4. Conclusions

The ability of *Psyllium* husk to form gel at lower temperatures was successfully employed in GF fresh pasta development with potential health benefits and lower GI. This approach led to the suppression of the flour pre-gelatinization step, a time/energy-consuming procedure. This is a strong argument for the industrial production of GF pasta. The optimized GF *Psyllium* pasta (50% *Psyllium* gel/50% rice flour) showed increased cooking and textural quality properties and carbohydrate IVD was in line with CRP and WP pastas. The *Psyllium* pasta showed very low lipid content, consistent with the low lipid content of the raw materials, and higher carbohydrate content than the other pastas, attributable to the higher healthy fibre content of *Psyllium*. Interestingly, all GF pastas showed significantly higher antioxidant activity than wheat pasta.

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Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

CRediT authorship contribution statement

Patrícia Fradinho: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Rita Soares:** Investigation. **Alberto Niccolai:** Investigation, Formal analysis, Writing - review & editing. **Isabel Sousa:** Supervision, Writing - review & editing, Funding acquisition. **Anabela Raymundo:** Conceptualization, Supervision,

Writing - review & editing.

References

Angus, A., & Westbrook, G. (2019). *Top 10 global consumer trends 2019*. London, UK: Euromonitor International. https://go.euromonitor.com/webinar-EC_2019-Global-Consumer-Trends-2019.html.

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næsa, T., et al. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International, 99*, 58–71.

Batista, A. P., Nunes, M. C., Fradinho, P., Gouveia, L., Sousa, I., Raymundo, A., et al. (2012). Novel foods with microalgal ingredients – effect of gel setting conditions on the linear viscoelasticity of Spirulina and *Haematococcus* gels. *Journal of Food Engineering, 110*, 182–189.

Belorio, M., Sahagún, M., & Gómez, M. (2019). *Psyllium* as a fat replacer in Layer cakes: Batter characteristics and cake quality. *Food and Bioprocess Technology, 12*, 2085–2092.

Berti, C., Riso, P., Monti, L. D., & Porrini, M. (2004). *In vitro* starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. *European Journal of Nutrition, 43*, 198–204.

Boisen, S., & Fernández, J. A. (1997). Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Animal Feed Science and Technology, 68*, 277–286.

Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W., & Delcour, J. A. (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition, 51*, 178–194.

Burešová, I., Tokár, M., Mareček, J., Hřivna, L., Faměra, O., & Šottníková, V. (2017). The comparison of the effect of added amaranth, buckwheat, chickpea, corn, millet and quinoa flour on rice dough rheological characteristics, textural and sensory quality of bread. *Journal of Cereal Science, 75*, 158–164.

Bustos, M. C., Perez, G. T., & León, A. E. (2013). Combination of resistant starches types II and IV with minimal amounts of oat bran yields good quality, low glycaemic index pasta. *International Journal of Food Science and Technology, 48*, 309–315.

Carlson, J. L., Erickson, J. M., Lloyd, B. B., & Slavin, J. L. (2018). Health effects and sources of prebiotic dietary fiber. *Current Developments In Nutrition, 2*(3), 1–8.

Chauvin, E. A. (2019). Health and wellness series – nutrition and women's health Global analysis report. *Agriculture and Agri-Food Canada*. http://www.agr.gc.ca/eng/industry-markets-and-trade/international-agri-food-market-intelligence/reports/health-and-wellness-series-nutrition-and-women-s-health/?id=1546614914143.

Crockett, R., Ie, P., & Vodovotz, Y. (2011). How do xanthan and hydroxypropyl methylcellulose individually affect the physicochemical properties in a model gluten-free dough? *Journal of Food Science, 76*(3), E274–E282.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356.

FDA. (2012). CFR – code of federal regulations title 21 [on-line]. U.S. Food and Drug Administration web site. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.81.

Fischer, M. H., Yu, N. X., Gray, G. R., Ralph, J., Anderson, L., & Marlett, J. A. (2004). The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk). *Carbohydrate Research, 339*(11), 2009–2017.

Foschia, M., Peressini, D., Sensidoni, A., Brennan, M. A., & Brennan, C. S. (2015). How combinations of dietary fibres can affect physicochemical characteristics of pasta. *LWT - Food Science & Technology, 61*, 41–46.

Foster-Powell, K., Holt, S. H. A., & Brand-Miller, J. C. (2002). International table of glycemic index and glycemic load values. *American Journal of Clinical Nutrition, 76*(1), 5–56.

Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M. R., et al. (2020). Effect of *Arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research, 45*, 101743.

Fradinho, P., Raymundo, A., Sousa, I., Domínguez, H., & Torres, M. D. (2019b). Edible Brown seaweed in gluten-free pasta: Technological and nutritional evaluation. *Foods, 8*(12), 622.

Fradinho, P., Sousa, I., & Raymundo, A. (2019a). Functional and thermorheological properties of rice flour gels for gluten-free pasta applications. *International Journal of Food Science and Technology, 54*(4), 1109–1120.

Ganesan, P., Kumar, C. S., & Bhaskar, N. (2008). Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresource Technology, 99*, 2717–2723.

Gasparre, N., & Rosell, C. M. (2019). Role of hydrocolloids in gluten free noodles made with tiger nut flour as non-conventional powder. *Food Hydrocolloids, 97*, Article 105194.

Gómez, M., & Martínez, M. M. (2016). Changing flour functionality through physical treatments for the production of gluten-free baking goods. *Journal Of Cereal Science, 67*, 68–74.

Haque, A., Richardson, R. K., & Morris, E. R. (1993). Xanthan-like "weak gel" rheology from dispersions of ispaghula seed husk. *Carbohydrate Polymers, 22*(4), 223–232.

de la Hera, E., Rosell, C. M., & Gomez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. *Food Chemistry, 151*, 526–531.

Jalali, M., Sheikholeslami, Z., Elhamirad, A. H., Khodaparast, M. H. H., & Karimi, M. (2020). The effect of the ultrasound process and pre-gelatinization of the corn flour on the textural, visual, and sensory properties in gluten-free pan bread. *Journal of Food Science & Technology, 57*(3), 993–1002.

Koh, L. W., Kasapis, S., Lim, K. M., & Woo, C. (2009). Structural enhancement leading to retardation of *in vitro* digestion of rice dough in the presence of alginate. *Food Hydrocolloids, 23*, 1458–1464.

Laleg, K., Barron, C., Santé-Lhoutellier, V., Walrand, S., & Micard, V. (2016). Protein enriched pasta: Structure and digestibility of its protein network. *Food & Function, 7*(2), 1196–1207.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering, 79*, 1033–1047.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Marsh, J. M., & Weinstein, D. B. (1966). Simple charring methods for determination of lipids. *Journal of Lipid Research, 7*, 574–576.

Mercier, S., Moresoli, C., Mondor, M., Villeneuve, S., & Marcos, B. (2016). A meta-analysis of enriched pasta: What are the effects of enrichment and process specifications on the quality attributes of pasta? *Comprehensive Reviews in Food Science and Food Safety, 15*, 685–704.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113–1124.

Niccolai, A., Zittelli, G. C., Rodolfi, L., Biondi, N., & Tredici, M. R. (2019). Microalgae of interest as food source: Biochemical composition and digestibility. *Algal Research, 42*, Article 101617.

Nunes, M. C., Batista, P., Raymundo, A., Alves, M. M., & Sousa, I. (2003). Vegetable proteins and milk puddings. *Colloids and Surfaces B: Biointerfaces, 31*, 21–29.

Obulesu, M., & Bhagya, S. (2006). Biochemical, functional and nutritional characteristics of soy protein concentrate prepared by thermal processing. *Journal of Food Science & Technology, 43*(2), 161–166.

Oh, K., Bae, I. Y., & Lee, H. G. (2014). *In vitro* starch digestion and cake quality: Impact of the ratio of soluble and insoluble dietary fiber. *International Journal of Biological Macromolecules, 63*, 98–103.

Palavecino, P. M., Ribotta, P. D., León, A. E., & Bustos, M. C. (2019). Gluten-free sorghum pasta: Starch digestibility and antioxidant capacity compared with commercial products. *Journal of the Science of Food and Agriculture, 99*(3), 1351–1357.

Parada, J., & Aguilera, J. M. (2011). Review: Starch matrices and the glycemic response. *Food Science and Technology International, 17*(3), 187–204.

Parada, J., Aguilera, J. M., & Brennan, C. (2011). Effect of guar gum content on some physical and nutritional properties of extruded products. *Journal of Food Engineering, 103*, 324–332.

Petitot, M., Abecassis, J., & Micard, V. (2009). Structuring of pasta components during processing: Impact on starch and protein digestibility and allergenicity. *Trends in Food Science & Technology, 20*(11-12), 521–532.

Pollet, A., Van Craeyveld, V., Van de Wiele, T., Verstraete, W., Delcour, J. A., & Courtin, C. M. (2012). *In vitro* fermentation of arabinoxylan oligosaccharides and low molecular mass arabinoxylans with different structural properties from wheat (*Triticum aestivum* L.) bran and psyllium (*Plantago ovata* forsk) seed husk. *Journal of Agricultural and Food Chemistry, 60*, 946–954.

Rafiq, A., Sharma, S., & Singh, B. (2017). Regression analysis of gluten-free pasta from brown rice for characterization and in vitro digestibility. *Journal of Food Processing and Preservation, 41*(2), Article e12830.

Rajauria, G., Jaiswal, A. K., Abu-Ghannam, N., & Gupta, S. (2013). Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed *Himanthalia elongata* from western coast of Ireland. *Journal of Food Biochemistry, 37*, 322–335.

Raymundo, A., Fradinho, P., & Nunes, M. C. (2014). Effect of Psyllium fibre content on the textural and rheological characteristics of biscuit and biscuit dough. *Bioactive Carbohydrates & Dietary Fibre, 3*, 96–105.

Samuelsen, A. B. (2000). The traditional uses, chemical constituents and biological activities of *Plantago major* L. - a review. *Journal of Ethnopharmacology, 71*, 1–21.

Singh, J., & Whelan, K. (2011). Limited availability and higher cost of gluten-free foods. *Journal of Human Nutrition and Dietetics, 24*, 479–486.

Storck, C. R., Zavareze, E. R., Gularte, M. A., Elias, M. C., Rosell, C. M., & Dias, A. R. G. (2013). Protein enrichment and its effects on gluten-free bread characteristics. *LWT - Food Science & Technology, 53*, 346–354.

Susanna, S., & Prabhasankar, P. (2013). A study on development of gluten free pasta and its biochemical and immunological validation. *LWT - Food Science & Technology, 50*, 613–621.

Thakur, V. K., & Thakur, M. K. (2014). Recent trends in hydrogels based on psyllium polysaccharide: A review. *Journal of Cleaner Production, 82*, 1–15.

Toutounji, M. R., Farahnaky, A., Santhakumar, A. B., Olic, P., Butardo, V. M., Jr., & Blanchard, C. L. (2019). Intrinsic and extrinsic factors affecting rice starch digestibility. *Trends In Food Science & Technology, 88*, 10–22.

Tudorică, C. M., Kuri, V., & Brennan, C. S. (2002). Nutritional and physicochemical characteristics of dietary fibre enriched pasta. *Journal of Agricultural and Food Chemistry, 50*, 347–356.

Vallons, K. J. R., Ryan, L. A. M., & Arendt, E. K. (2011). Promoting structure formation by high pressure in gluten-free flours. *LWT - Food Science & Technology, 44*, 1672–1680.

Van Craeyveld, V., Delcour, J. A., & Courtin, C. M. (2008). Ball milling improves extractability and affects molecular properties of psyllium (*Plantago ovata* forsk) seed husk arabinoxylan. *Journal of Agricultural and Food Chemistry, 56*, 11306–11311.
Vici, G., Belli, L., Biondi, M., & Polzonetti, V. (2016). Gluten free diet and nutrient deficiencies: A review. *Clinical Nutrition, 35*, 1236–1241.
Ziemichód, A., Wójcik, M., & Różyło, R. (2019). Seeds of *Plantago psyllium* and *Plantago ovata*: Mineral composition, grinding, and use for gluten-free bread as substitutes for hydrocolloids. *Journal of Food Process Engineering, 42*, Article 12931.

Chapter 4. Study and processing of *Laminaria ochroleuca* for gel applications

The work presented in this chapter was included in the following publications:

- ***Technical Review***

  Torres, M.D., Fradinho, P., Domínguez, H. (2018). New life for marine, agricultural and forestry by-products - personal care hydrogels with functional features. *H&PCToday*, 13(6), 7-11. https://www.teknoscienze.com/wp-content/uploads/2018/11/Torres.pdf

- ***Research Paper 3***

  Fradinho, P., Flórez-Fernández, N., Sousa, I., Raymundo, A., Domínguez, H., Torres Pérez, M.D. (2020). Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties. *Journal of Applied Phycology*, 131, 109787. https://doi.org/10.1007/s10811-019-01958-8 (Impact Factor: 2.635, Q1)

- ***Research Paper 4***

  Fradinho, P., Raymundo, A., Sousa, I., Domínguez, H., Torres Pérez, M.D. (2019). *Psyllium* and *Laminaria* partnership – an overview of possible food gel applications. *Applied Sciences*, 9(20), 4356. https://doi.org/10.3390/app9204356 (Impact Factor: 2.217, Q2)

In Figure D the schematic procedure of chapter 4 is presented.

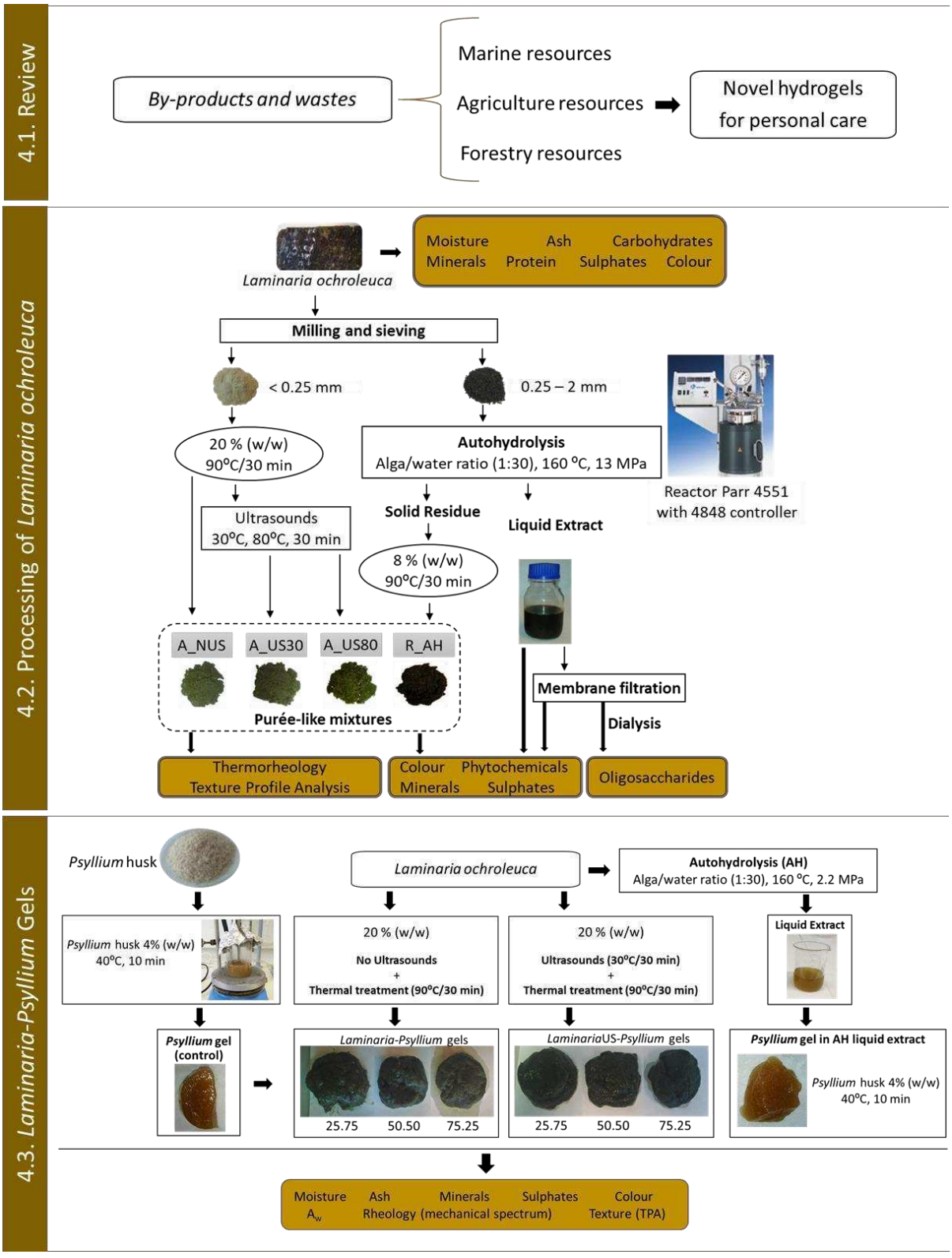

Figure D. Schematic procedure of chapter 4. A_NUS (Alga 20%, 90°C/30 min), A_US30 (Alga 20%, 90°C/30 min, US 30°C/30 min), A_US80 (Alga 20%, 90°C/30 min, US 80°C/30 min), R_AH (autohydrolysis residue), and US (ultrasound treatment).

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New life for marine, agricultural and forestry by-products – personal care hydrogels with functional features

KEYWORDS: Wastes, marine, agricultural, forestry, biopolymers, functional features, novel formulations.

ABSTRACT

This short review, covering scientific papers published from 2014 until now, deals with the use of by-products from the marine, agricultural and forestry resources for the development of novel hydrogels with functional features for personal care applications, with the consequent added value. An overview about natural matrices based on hydrogels for other applications was also given. Some representative examples of residual raw materials, their mechanical and functional properties was summarised in this review. The last part covers the future applications for these emerging matrices, since consumers look for natural and sustainable personal care products.

INTRODUCTION

The move toward green, sustainable, natural, functional products has been growing in the cosmetic and personal care industry (1). In the last decade, the amount of waste deriving from industrial processes has risen substantially. Many industries produce different types of disposable by-products, rich in valuable compounds. Their characterization and valorisation could not only convert them into high value products with application in diverse biotechnological fields, such as personal care, pharmaceutics, biomedical, food or cosmetic, but would also reduce the disposal environmental impact and the related treatment costs (2). Therefore, the global marine, agricultural and forestry industries have aimed its vision towards the integrated biorefinery. The goal is to produce diversified, innovative and revenue generating product lines using on-site bioresources. The continuous development of new bioactive or gelling ingredients for personal care and cosmetic products is one of the most important areas of research in these industries.The academia is ahead of the issued guidelines – several studies have been conducted around this matter (see as e.g. 3, 4). There are many examples of effective, inexpensive and bio-sustainable materials, and thus represent a valid alternative to the regular plant derived extracts, more commonly adopted in personal care formulations.

Consumers look for natural products, so that various plant extracts that formed the basis of medical treatments in ancient civilizations are still used nowadays in cleaners, astringents, moisturizers, and many other skin care products.

Hydrogels (*i.e.* water based gels) have become very popular due to their unique properties such as high water content, softness and flexibility (2, 5). Compared with the traditional polymer hydrogel materials, bio-based hydrogels are getting more attention for the consumer, because of its environmentally friendliness, biocompatibility, special functional and biodegradable advantages, reducing the dependence on fossil fuels (6). Rising number of scientific publications on hydrogels applications is a proof of the relevance of this research topic (Figure 1). The major areas of hydrogel applications are contact lenses, drug delivery systems, wound dressings, tissue engineering, or hygiene products. On the personal care market, products in many hydrogels forms are available, including skin care hydrogel and hydrogel masks or eye-pads.

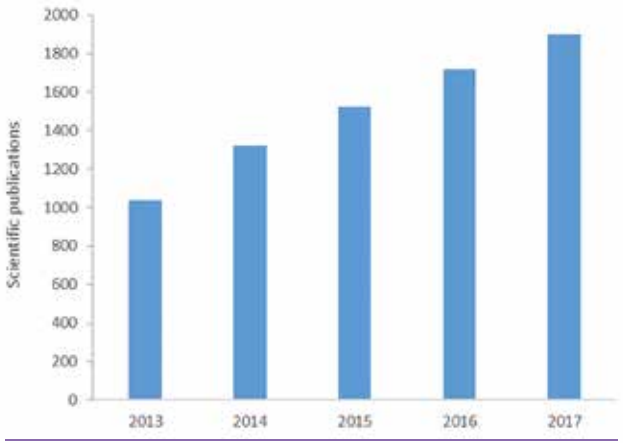

Figure 1. Scientific publications on hydrogels applications in the last years (scopus database, august 2018). Note here that bars correspond to primary y-axis.

In this context, enhancing the functional and mechanical features of bio-based hydrogels formulated with by-products from different sources in personal care products remains a relevant task for research and development, which is a concomitant challenge towards the biorefinery concept and circular economy. Hence, the main aims of this short review, covering scientific papers from 2014 until now, are to outline the present status and future trends on functional hydrogels based on by-products from marine, agricultural or forestry sources for the development of personal care products.

	Resource	Raw material	Mechanical/ Functional properties	Possible final personal care application	Reference
Marine	Microalgae, *Isochyrsis sp.*	Wax (*i.e.*, long-chain alkenones)	Stable for 10 weeks	Lipsticks and lip balms	1
	Shells	Chitosan-based hydrogels	Superabsorbent features	Personal hygiene hydrogels	2
	Red seaweeds	Carrageenan-based hydrogels	Gel strength, antimicrobioal activity	Mucoadhesive hydrogels for drug delivery	8
	Brown seaweeds	Alginate-based hydrogels	Gel-like and stable nanocarriers	Topical hydrogels	7
	Fishes and invertebrate marine animals	Collagen-based hydrogels	High moisturizing action, anti-aging, anti-wrinkling	Anti-aging hydrogels, UV radiation protectors	6
Agricultural	Rice, *Oryza sativa*	Rice water	Anti-aging efficacy, skin care	Skincare hydrogel	5
	Chestnut, *Castanea sativa*	Bur	Antioxidants; skin-oxidative stress	Topical hydrogels	13
	Coffee silverskin	Caffeine	Nanostructured lipid carriers	Topical hydrogels for cellulitis	12
	Potato, *Solanum tuberosum*	Peel	Antioxidant, antidiabetic features	Starch-based hydrogels for skin care	11
	Starchy sources	Starch-based hydrogels	Adsorbent features	Water-insoluble hydrogels for disposable diapers	10
Forestry	Cellulosic plants	Cellulose-based hydrogels	Superabsorbent features	Diapers, panty liners, tampons, paper towels, and tissue paper	18
	Land plants	Pectine-based hydrogels	Emulsifier, gelling properties	Skin care gelling lotions	17
	Oak, *Quercus robur Laciniata*, and pine, *Pinus pinaster Aiton*	Ashes	Gel-like and skin care	Topical potassium soaps	16
	Tea tree	Oil	Antiedematogenic, skin wound healing features	Topical skin hydrogels, soaps, gel-creams	15
	Ligno-cellulosic plants	Lignin-based hydrogels	Antioxidant, adsorbent features	Hydrophobic drug delivery hydrogels	14

Table 1. By-products from representative natural sources used to personal care based products reported in the last five years.

MARINE RESOURCES

Marine organisms and algae are rich in bioactive and gelling compounds currently underused that could be suitable for personal care products.There is a notable number of new personal care hydrogels based on this novel generation of bioactive ingredients. Some representative examples are presented in Table 1. In a comprehensive review, Silva and co-workers (6) detailed the studies that has been made regarding collagen isolation from different marine resources (*i.e.* fishes, or invertebrate marine animals, such as jellyfish or marine sponges) and its further use in health care applications. Its characteristics take to the development of hydrogels with not only high moisturizing action, but also other activities such as anti-wrinkling, anti-aging or UV radiation protectors. Nguyen and co-workers (7) proposed the use of brown seaweeds to develop topical alginate-based hydrogels incorporated with curcumin. The authors evaluated the possibility of designing an innovative nano-carrier of hydrophobic bioactive molecules and determining its potential as an ingredient for a topical hydrogel. Padhi and co-workers (8) reported carrageenan-based hydrogels featuring not only mechanical strength but also additional water retention, hemocompatibility, mucoadhesivity and antimicrobial activity. Cheng and co-workers (2) proposed chitosan-based superabsorbent hydrogels with promising applications for personal hygiene products.

McIntoch and co-workers (1) found that wax sourced from marine microalgae *Isochrysis* biomass exhibited potential application in lip balms or lipsticks. These authors indicated the potentiality of these bioactive compounds for sunscreen applications. These innovative approaches towards inexpensive sources of rich bioactive compounds could be very valuable alternatives to personal care, cosmetic or health care markets.

AGRICULTURAL RESOURCES

The agricultural processing industries produce from 10 to 60% of solid wastes such as skins, stems, seeds, leaves, waste waters and unusable pulp which are normally discarded although in some cases they can present more valuable potentiality than the main products (9). Table 1 illustrates some representative example of by-products from agricultural resources with potential to develop personal care hydrogels. In a comprehensive review (10), the potentiality of starch-based hydrogels for personal care applications was explained. Later authors indicated that water-insoluble starch hydrogels are capable of absorbing a large amount of fluids making them suitable for films in disposable diapers. Niphadkar and co-workers (11) proposed starch-based hydrogels incorporated with bioactive compounds present in the potato peel for the development of skin care formulation.

Rodrigues and co-workers (12) recommended caffeine extracts from coffee silverskin, which possess properties that would render them as a promising alternative for the topical treatment of cellulite. Pinto and co-workers (13) reported *Castanea sativa* by-products, mainly burs, as promising bioactive ingredients for the formulation of topical hydrogels. Marto and co-workers (5) suggested the rice (*Oryza sativa*) water, a food processing waste, as an interesting ingredient in skincare hydrogels. These authors designed and developed a topical gel formulation containing rice water with anti-aging and antioxidant properties. All above-mentioned high value compounds can be incorporated in hydrogels with beneficial health effects in skin-care formulations contributing to the advance of the agriculture industry and consequently to the economic and environmental sustainability.

FOREST RESOURCES

Forest residues such as wood, bark, peat, humus or plant-based extracts can be also used as organic raw materials in various health care and cosmetic hydrogels (Table 1). Mckeen and co-workers (14) described a green procedure to prepare lignin-based hydrogels with antimicrobial activities. These authors also highlighted the potential biomedical applications of these lignocellulosic hydrogels. Flores and co-workers (15) showed the feasibility to prepare semisolid formulations (hydrophilic hydrogels) containing *M. Alternifolia* essential oil from tea tree.

The proposed hydrogels presented satisfactory features compatible to topical application. Torres and Seijo (16) studied the potential of wood ashes from oak, *Quercus robur Laciniata*, and pine, *Pinus pinaster Aiton* to develop topical potassium soaps. In a comprehensive review, Noreen and co-workers (17) presented the pectin-based hydrogels as a non-toxic, biocompatible and biodegradable source for the formulation of skin care gelling lotions. Bashari and co-workers (18) studied cellulose-based hydrogels as superabsorbent matrices. The excellent biocompatibility of cellulose has prompted the large use of cellulose-based personal care hydrogels (*i.e.* diapers, panty liners, tampons, paper towels, or tissue papers). The performance and price when compared with their conventionalcounterparts remains a significant challenge for cellulose-based personal care hydrogels. Even though, the increasing demand emerging for biodegradable hydrogels made from renewable resources for personal care applications could be alleviated with the proposed hydrogels above-mentioned.

CONCLUSIONS AND FUTURE TRENDS

To conclude, it should be pointed out that there is a broad range of disposable by-products from agricultural, marine and forest sources, rich in high-valuable compounds that could be attractive alternatives to personal care, cosmetic or health care applications.

The future perspectives for personal care products are placed on the development of personalized bio-based hydrogels. These materials already have a well-established role in hygiene products, contact lenses, or wound dressing markets but commercial personalized hydrogels as matrix for smart products, designed for a specific type of skin, that react to pH, moisture changes, oxidative stress, among others, are still limited. The challenge is to prepare commercial competitive hydrogels formulated with biopolymers from by-products that deliver active compounds. The development of high performance hydrogels for personal care products could be overcome by the use of emerging technologies as 3D printing, whose potential has not been fully explored yet. Overall, challenges that will need to be addressed include management of suitable raw materials, performance of bio-based hydrogels, and their cost for production. Economy of scale will be one of the most relevant challenges for the production of functional bio-based hydrogels from underused natural sources.

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REFERENCES

1. McIntosh, K., Smith, A., Young, L.K., Leitch, M.A., Tiwari, A.K., Reddy, C.M., O'Neil, G.W., Liberatore, M.W., Chandler, M., Baki, G. (2018). Alkenones as a promising green alternative for waxes in cosmetics and personal care products. Cosmetics, 5, 34-47.
2. Cheng, B., Pei, B., Wang, Z., Hu, Q. (2017). Advances in chitosan-based super absorbent hydrogels. RSC Advances, 7, 42036–42046.
3. STOA (Science and Technology Options Assessment) (2013). *Recycling agricultural, forestry & food wastes and residues for sustainable bioenergy and biomaterials*. Institute for European Environmental Policy.
4. Balboa, E.M., Soto, M.L., Nogueira, D.R., González-López, N., Conde, E., Moure, A., Vinardell, M.P., Mitjans, M., Domínguez, H. (2014). Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics. Industrial Crops and Products, 58, 104-110.
5. Marto, J., Neves, A., Gonçalves, L.M., Pinto, P., Almeida, C., Simões, S. (2018). Rice water: a traditional ingredient with anti-aging efficacy. *Cosmetics*, 5, 26-38.
6. Silva, T.H., Moreira-Silva, J., Marques, A.L.P., Domingues, A., Bayon, Y., Reis, R.L. (2014). Marine origin collagens and its potential applications. Marine Drugs, 12, 5881-5901.
7. Nguyen, H.T.P., Munnier, E., Souce, M., Perse, X., David, S., Bonnier, F., Vial, F., Yvergnaux, F., Perrier, T., Cohen-Jonathan, S., Chourpa, I. (2015). Novel alginate-based nanocarriers as a strategy to include high concentrations of hydrophobic compounds in hydrogels for topical application. Nanotechnology, 26, 255101-255114.
8. Padhi, J.R., Nayak, D., Nanda, A., Rauta, P.R., Ashe, S., Nayak, B. (2016). Development of highly biocompatible Gelatin & i-Carrageenan based composite hydrogels: In depth physiochemical analysis for biomedical applications. Carbohydrate Polymers, 153, 292-301.
9. Barbulova, A., Colucci, G., Apone, F. (2015). New trends in cosmetics: by-products of plant origin and their potential use as cosmetic active ingredients. *Cosmetics*, 2, 82-92.
10. Ismail, H., Irani, M., Ahmad, Z. (2014). Starch-based hydrogels: present status and applications. International Journal of Polymeric Materials and Polymeric Biomaterials, 62, 411–420.
11. Niphadkar, S.S., Vetal, M.D., Rathod, V.K. (2015). Purification and characterization of polyphenol oxidase from waste potato peel by aqueous two-phase extraction. Preparative Biochemistry and Biotechnology, 45, 632-649.
12. Rodrigues, F., Alves, A.C., Nunes, C., Sarmento, B., Amaral, M.H., Reis, S., Oliveira, M.B.P.P. (2016). Permeation of topically applied caffeine from a food by-product in cosmetic formulations: Is nanoscale in vitro approach an option? International Journal of Pharmaceutics, 513, 496–503.
13. Pinto, D., Braga, N., Rodrigues, F., Oliveira, M.B.P.P. (2017). *Castanea sativa* bur: an undervalued by-product but a promising cosmetic ingredient. *Cosmetics*, *4*, 50-65.
14. McKeen, L. W. (2014). 3-Plastics used in medical devices. In: Handbook of polymer applications in medicine and medical devices; Modjarrad, K., Ebnesajjad, S., Eds.; William Andrew Publishing: Oxford, 21□53.
15. Flores, F.C., De Lima, J.A., Da Silva, C.R., Benvegnú, D., Ferreira, J., Burger, M.E., Beck, R.C.R., Rolim, C.M.B., Rocha, M.I.U.M., Da Veiga, M.L., Da Silva, C.D.B. (2015). Hydrogels containing nanocapsules and nanoemulsions of tea tree oil provide antiedematogenic effect and improved skin wound healing. Journal of Nanoscience and Nanotechnology, 15, 800-809.
16. Torres, M.D., Seijo, J., 2016. By-products from the chestnut industry used to produce natural potassium soaps: physicochemical properties. Journal of Surfactants and Detergents, 19, 381-387.
17. Noreen, A., Nazli, Z.-i-H., Akram, J., Rasul, I., Mansha, A., Yaqoob, N., Iqbal, R., Tabasuma, S., Zuber, M., Zia, K.M. (2017). Pectins functionalized biomaterials; a new viable approach for biomedical applications: A review. International Journal of Biological Macromolecules, 101, 254–272.
18. Bashari, A., Rouhani Shirvan, A., Shakeri, M. (2018). Cellulose-based hydrogels for personal care products. Polymers for Advanced Technologies, DOI: 10.1002/pat.4290.■

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Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties

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Abstract

Dehydrated *Laminaria ochroleuca* was processed by autohydrolysis with compressed hot water to extract bioactive compounds. Both the whole algae and individual fractions obtained (solid residue and liquor) were characterised to assess its functional properties for future innovative food applications. Purée-like systems were developed by combining ultrasonic and thermal technologies to maximise the antioxidant capacity and were evaluated by determining colour, texture, rheology, syneresis and the presence of bioactive compounds. Overall, the obtained results indicated that *L. ochroleuca* is a valuable resource that can be used as a whole or taking advantage of its bioactive fractions, in a concept of circular economy and sustainability.

Keywords Phaeophyta · Edible brown seaweed · Autohydrolysis · Ultrasounds · Waste · Antioxidants

Introduction

Widely consumed in Asian countries, the edible brown algae *Laminaria ochroleuca* (also known as kombu) is mainly valued in Europe through its extracts, which include alginates, bioactives, and pigments (Fernandes et al. 2016). Alginates are commercially used as thickening, gelling, and stabilising agents in food, cosmetic and pharmaceutical industries. This alga also contains fucoidans that reduce the expression of the pro-inflammatory cytokines and have antioxidant, antimicrobial, and antitumoral properties (Flórez et al. 2017). An alternative could be the development of enriched foods with texture appropriate to special groups of the population, since, by 2050, 1/5 of the world's population will be over 60 years old, numbers that increase to more than 30% in Europe (WHO 2015). Brown algae present a chemically and structurally complex cell wall composed of sulphated and branched polysaccharides (fucoidan) associated with proteins and various bound ions (e.g. Ca, K) that limits the efficient extraction of the intracellular and wall compounds (Kadam et al. 2015a).

The use of green technologies such as autohydrolysis (AH) and ultrasound-assisted extraction (UAE) could be valuable tools to extract the functional components of this brown alga, towards its integral valorisation. Water-based extraction is food compatible, non-expensive, and environmentally friendly but has low selectivity with low extraction efficiency (Flórez-Fernández et al. 2019). Namely, AH can be performed operating under high temperature and pressure extraction conditions, changes water properties, allowing solubilisation and depolymerisation of compounds present in the matrix of the seaweed (González-López et al. 2012). Since UAE is a useful technique to enhance extraction of bioactive compounds from brown algae (Kadam et al. 2015b), a combination of both techniques mentioned above could be adequate to enhance the yields of polysaccharides with antioxidant and glycosidase inhibitory activities. UAE could provide an economically feasible technique with potential for scale-up and suited for thermolabile compounds (Wan et al. 2015).

Several studies have been performed with *L. ochroleuca* extracts (López-Hortas et al. 2018). However, little is known about the whole algae functional properties, apart from some studies regarding its chemical composition (Sánchez-Machado et al. 2004). In this context, this study aims at the integral valorisation of *L. ochroleuca* using environmentally friendly technologies for the recovery of functional compounds. The chemical and phytochemical properties of the

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obtained extracts as well as the mechanical features of the formulated systems with the whole alga were performed. Potential applications for both extracts and solid residual fractions were outlined.

Materials and methods

Materials

Dehydrated *Laminaria ochroleuca* used as raw material was generously provided by Algas Atlánticas Algamar Co. (Pontevedra, Spain). Algae were milled and sieved in order to obtain particle size ranges below 0.25 mm, and between 0.25 and 2.0 mm. For comparison purposes, two commercial references were used, namely, a purée-like food product aimed for the senior population and fresh pasta with spinach filling.

Subcritical water extraction treatment

The coarse milled fraction (0.25–2 mm) *L. ochroleuca* was subjected to hydrothermal processing (i.e. autohydrolysis) with compressed hot water at 160 °C, using a liquid:solid ratio of 30:1 (w/w), to isolate fucoidan and phlorotannin fractions from the alga (Flórez-Fernández et al. 2019). Four extraction trials were made by placing around 60 g (d.b.) of algae in a pressurised reactor (Parr 4848, USA), operating at about 7.5 atm. Then, a conventional filtration process was used to separate the solid and liquid phases. Both liquor and solid residue were chemically characterised and used to prepare purée-like mixtures (Fig. 1). The yield of each extraction trial was calculated, as well of severity factor (log Ro), i.e. the treatment efficiency, according to Overend and Chornet (1987). The molecular weight cut-off (MWCO) of the liquid phase was performed using an Amicon stirred cell (model 8400, Millipore), using membranes of 100, 50, and 30 kDa. All separated fractions (> 100, 50–100, 30–50, and < 30) were stored at 4 °C until further use.

Preparation of the purée-like mixtures

The lowest particle size fraction of *L. ochroleuca* (< 0.25 mm) was used to prepare aqueous dispersions based on the results found for other biopolymer-based materials (e.g. Moreira et al. 2014). Several preliminary trials were conducted to find the optimal preparation conditions: alga sample (10–20% w/w, d.b.) was dispersed in water (40–90 °C), under mechanical stirring (Eurostar Digital, IKA-WERKE) at 300 rpm during 10–30 min. The solid residue from AH was thermally processed in the optimal conditions defined for alga samples. For UAE, an ultrasonic bath (P120H, Elma, Germany) operating at 80 Hz for 10–30 min was used and two temperatures (30 and 80 °C) were tested (Fig. 1). In order to not disturb the formation of the matrix, the samples were prepared directly in individual sealed glass containers (35 mm height, 32 mm diameter), and left at 5 °C for 24 h to ensure full maturation.

Chemical measurements

Centesimal composition

Moisture and ash contents (dry basis, d.b.) were determined by gravimetric methods after drying samples at 105 ± 2 °C and incinerating (575 °C, 6 h), respectively. The nitrogen content was assessed by mass spectrometry using a FlashEA 1112 elemental analyser (Thermo Fisher Scientific). A N-protein conversion factor of 5.38 for brown algae (Lourenço et al. 2002) was used to obtain the protein content (d.b.) of the samples.

Mineral and metals content

Mineral and metal contents were determined after microwave-assisted (SAVILLEX) acid digestion (80 °C, 6 h). Ca, Mg, K, Na, P, As, Cu, Cd, Pb, Fe, and Zn were analysed by Inductively Coupled Plasma Optical Emission Spectrometry (Optima 4300 DV, Perkin Elmer). Iodine was determined after digestion with tetramethylammonium hydroxide (80 °C, 6 h) by Inductively Coupled Plasma Mass Spectrometry (X Series ICP-MS, Thermo Elemental). Mercury determination was made using Cold Vapor Atomic Absorption Spectrometry according to Fernández-Fernández et al. (2007).

Sulphate content

Sulphate content was determined at least in triplicate by two different methods. For liquid samples, gelatin-barium chloride procedure was used (Dodgson 1961). Absorbance was measured (500 nm) after incubation at 25 °C, using a standard curve made with K_2SO_4. For solid samples, sulphate content was obtained by ionic chromatography method (mobile phase: 3.2 mM sodium carbonate/1 mM sodium bicarbonate at 0.70 mL min^{-1}) as previously reported (Gómez-Ordóñez et al. 2010).

Carbohydrate content

Alga (0.25–2 mm) was hydrolysed with H_2SO_4 (72 % v/v) in a water bath (30 °C, 60 min). Subsequently, samples were hydrolysed with sulphuric acid (4% v/v) in an autoclave (121 °C, 60 min), and the resulting samples were analysed using High-Performance Liquid Chromatography (HPLC) (Flórez-Fernández et al. 2017).

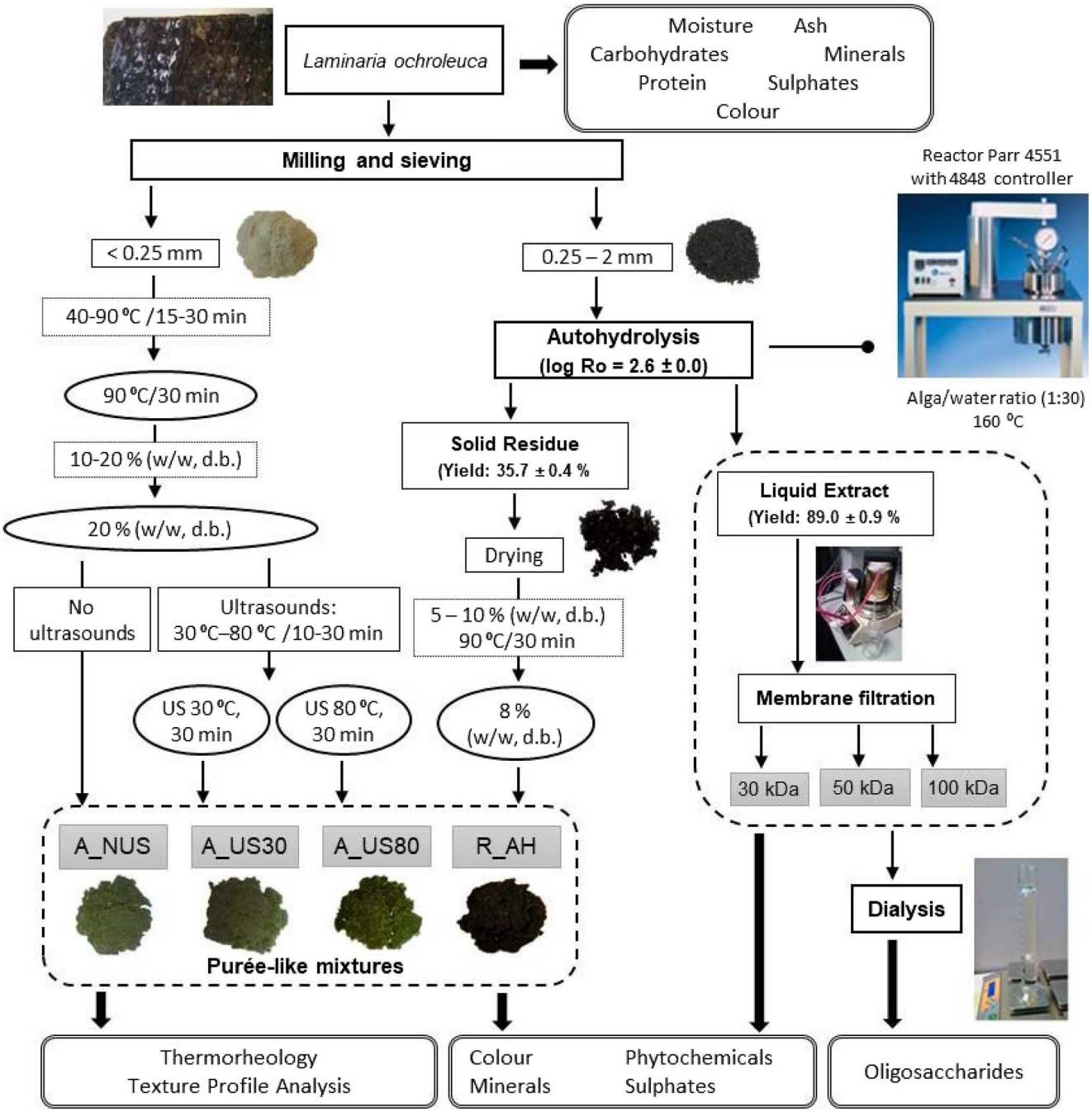

Fig. 1 Schematic representation of the present study, showing the preliminary assayed conditions (squares), the optimal conditions adopted (round), and the characterisation performed (double line). The pictures depict *Laminaria ochroleuca* and its milled fractions, the autohydrolysis reactor and AH products (solid residue and liquor), the dialysis procedure and the end-products (purée-like mixtures). A_NUS (Alga 20%, 90 °C/30 min), A_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min)

Oligosaccharides determination

The oligosaccharides in the liquid extract were determined by HPLC. First, the salt content in the samples was reduced to 0.2 μS cm^{-1} by dialysis using a membrane tubing (Spectra/Por, Float-A-Lyzer G2, MwCo: 100-500 Da). Then, extracts were filtered through 0.45-μm membranes. Glucose, fucose, rhamnose, formic acid, and acetic acid were analysed using 300 × 7.8 mm Aminex HPX-87H column (BioRad, USA), operating at 60 °C with sulphuric acid (0.003 M, 0.6 mL min^{-1}) as mobile phase.

High-performance size-exclusion chromatography (HPSEC)

HPSEC was performed to determine the molar mass distribution of the samples, using two 300 × 7.8 mm TSK-Gel

columns in series (G3000PWXL and G2500PWXL, Tosoh Bioscience, Germany) operating at 70 °C, and a 40 × 6 mm PWX-guard column. Milli-Q water with a 0.4 mL min^{-1} flow rate was employed as mobile phase. Dextrans below 80,000 g mol^{-1} were used as calibration standards.

Instrumental colour

All measurements were performed using a Minolta CR-400 (Japan) tristimulus colorimeter (five replicates). The colour parameters (L^*, a^*, and b^*) were assessed by CIELAB, at 20 ± 1 °C under the same light conditions. L^* represents the lightness, a^* the redness or greenness degree, and b^* the yellowness or blueness degree. The total colour differences (ΔE^*) between samples were determined according to Expression (1):

$$\Delta E^* = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 \right]^{1/2} \quad (1)$$

Texture

The texture analysis of the samples was conducted on a texturometer TA-XT2 (Stable MicroSystems, UK). Texture profile analysis was done in a double penetration mode using a P0.25S stainless steel probe (5 kg load cell, 12 mm penetration depth at 1.0 mm s^{-1} crosshead speed). From the texturograms (i.e. force versus time plots), the parameters with the greatest ability to discriminate the samples texture were firmness (N, maximum resistance to the penetration of the probe), cohesiveness (how well the system structure withstands penetration), and adhesiveness (N.s, resistance of the material when the probe is recessing). All measurements were performed five times at 20 ± 1 °C.

Thermorheology

Small amplitude oscillatory shear testing was conducted at least in duplicate, and storage (G′) and loss (G″) moduli were employed to follow the viscoelastic features of purée-like mixtures during the formation process. Suspensions of selected systems were prepared according to the procedure already described and immediately placed into the bottom plate of a controlled stress rheometer (RheoStress 600, Haake, Germany) using cone-plate geometry (diameter 35 mm and angle 2°) and covered with paraffin oil to prevent water loss.

Samples were equilibrated (5 min, 25 °C) before rheological testing. Firstly, stress sweep tests (0.1 to 100 Pa) were run at 1 Hz, and 25 and 90 °C, and the linear viscoelastic region selected: 0.1 to 35 Pa for aqueous seaweed solutions and 0.1 to 65 Pa for purée-like systems. Afterwards, the rheological testing consisted in the following procedure: (1) heating ramp from 25 to 90 °C (2 °C min^{-1}, 1 Hz, 15 Pa) to define the sample melting behaviour; (2) temperature setting (90 °C, 15 min); (3) cooling ramp from 90 to 5 °C (1 °C min^{-1}, 1 Hz, 15 Pa) to follow the viscoelastic evolution with temperature; (4) time sweeps (5 °C, 60 min, 1 Hz, 30 Pa) to corroborate the sample maturation kinetics; (5) frequency sweep from 0.1 to 100 Hz (5 °C, 30 Pa) to assess the mechanical spectrum of the sample.

Bioactive compounds

Five grams of purée-like mixtures were mixed with 95 g of distilled water and incubated at 90 °C for 1 h. After extraction, dispersions were centrifuged (3000×g, 10 min, room temperature) and the supernatant was used as extract. Liquid samples were used as it is. Dry weight of all extracts was determined in order to express the bioactive compounds in the solid samples. The following spectrophotometric methods were performed in an Evolution 201 UV-Vis Spectrophotometer (ThermoScientific), and all analyses were carried out at least in triplicate.

Scavenging activity

The ABTS radical cation ($ABTS^{\cdot+}$) [2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate)] scavenging capacity was determined according to Re et al. (1999). Samples (20 μL) and TEAC solution (2 mL) were mixed and incubated (30 °C, 6 min). Phosphate-buffered saline was used as blank. The corresponding absorbance was measured at 734 nm and the results were expressed as equivalents of Trolox (mM).

Phloroglucinol content

Phloroglucinol content was determined by the Folin–Ciocalteu method as reported by Koivikko et al. (2005). Briefly, sample (500 μL), Folin–Ciocalteau (500 μL, 1 N) and Na_2CO_3 (1 mL, 20% w/w) were mixed and incubated (45 min in the dark) at 25 °C. Afterwards, the mixture was centrifuged (1600×g, 8 min) before measuring the absorbance at 730 nm.

Statistical treatment

Statistical analysis was performed using one-way ANOVA (Statistica, version 10.0, StatSoft Inc., USA). Whenever the former analysis exhibited differences amongst means, a Tukey test was conducted to differentiate means with 95% confidence ($p < 0.05$).

Results and discussion

Schematic procedure

The schematic procedure adopted in this study is depicted in Fig. 1. Commercial dried *Laminaria ochroleuca* was milled and sieved into two particle size fractions. The one with larger particle size (0.25–2 mm) was used for subcritical water extraction (autohydrolysis) using previously optimised conditions for this alga taking into account the maximum bioactive compounds concentration obtained in the liquid extract (Flórez-Fernández et al. 2019). The smallest particle size fraction (< 0.25 mm) was used to study the effect of different processing treatments on the production of an algae-based structure. Preliminary trials were performed with alga concentrations from 10 to 20% (w/w, d.b.) and temperatures from 40 to 90 °C applied for 15 and 30 min. The best conditions—20%, 90 °C, 30 min—were selected based on the absence of syneresis and in the formation of a visually compact structure that does not flow. According to Kadam et al. (2015b), UAE can be employed to enhance the extraction of bioactive compounds from seaweed. So, preliminary trials were performed applying UAE at 30 and 80 °C for 10 and 30 min. Three purée-like mixtures were selected for further analysis: A_NUS (Alga 20%, 90 °C/30 min), A_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min).

On the other hand, from AH processing, two fractions were obtained: a liquid extract (liquor) and a solid residue. Traditionally, only the liquid extract is used, and the solid residue discarded. However, this study intends to valorise the entire algae and its AH fractions, in an economically sustainable logic reinforced by the European Community (EC) directive on circular economy (EC 2015a). So, the solid fraction obtained from AH was characterised and possible applications were studied. The solid residue was dried at 60 °C for 70 h for conservation purposes, and then preliminary trials were performed using 5 to 10% (w/w, d.b.) residue mixed with water and thermally processed in the same way as alga samples. The selected formulation was 8% (w/w, d.b), 90 °C/30 min (R_AH). Concerning the liquid AH fraction (liquor), it was purified using membranes with different MWCO: 30 kDa, 50 kDa, and 100 kDa based on the results previously obtained for other brown seaweeds (Álvarez-Viñas et al. 2019).

Physicochemical and phytochemical characterisation of *L. ochroleuca* and autohydrolysis fractions

The distinct feature of *L. ochroleuca* is its high ash content (Table 1). The mineral content of seaweeds varies according to seaweed species, but other factors also play a relevant role, like oceanic residence time, the geographical place of harvest, wave exposure, seasonal, annual, environmental and physiological factors, type of processing, and method of mineralisation (Rupérez 2002). Table 1 values are consistent with those reported by Rupérez (2002) and Sánchez-Machado et al. (2004). As compared with other marine species, *Laminaria* spp. have greater ability to extract minerals from the seawater and hence accumulate high amounts of some elements, such as magnesium, calcium, and iodine (Kim and Bhatnagar 2011), presenting here an opportunity for the development of food products with low sodium content as already reported for beef patties with *Undaria pinnatifida* (López-López et al. 2011).

Regarding dried *L. ochroleuca* protein content, the value found is consistent with Sanchez-Machado et al. (2004) results for the same alga species (7.49%, d.b.). Generally, protein content of seaweeds is low when compared with polysaccharides and polyphenols; therefore, they are rarely valued for the functional properties of their proteins (Conde et al. 2013). Amongst seaweeds, Phaeophyta present the lowest protein values (6.4–19.8%, d.b.), although it can vary according to the factors described earlier and the analytical methods used (Černá 2011). It is noteworthy that the obtained value for the dried seaweed is lower than the sum of the AH fractions, which can be due to low extraction efficiency in alga sample because of its complex polysaccharide cell wall structure. During subcritical water extraction, proteins are degraded and the water soluble peptides could be extracted (Castro-Puyana et al. 2013) and therefore be present in the AH liquor.

Regarding the solid residue obtained from AH, it still maintains some valuable compounds, namely minerals (K, Ca, and Na). In brown algae, sulphate is a component of fucoidan, a sulphated polysaccharide present in the cell wall of these organisms that protect the algae from desiccation (Rupérez 2002). The complex composition of fucoidans remains uncertain despite the numerous studies about their composition and chemical structure (Martínez-Hernández et al. 2017). It is noteworthy that AH processing promoted the solubilisation of the sulphates since liquor shows a higher content than that of dried *Laminaria*.

Apart from bioaccumulating essential minerals such as Ca, Mg, Fe, and I, seaweeds can also concentrate other elements that can represent a health risk for consumers, namely Pb, Cd, and As. Indeed, due to the adsorption capacity of seaweeds, several studies have been conducted aiming for wastewater treatment and pollution control (Lodeiro et al. 2005). East Asian cultures have traditionally exploited *Laminaria* spp. for food and medicine, and this alga is even listed in the Chinese pharmacopoeia (Kim and Bhatnagar 2011). Unexpectedly, in these countries, which are responsible for most seaweeds' production and consumption as human food, no specific limits have been established regarding its toxic metals content (Circuncisão et al. 2018). In Western countries,

Table 1 Chemical composition of *L. ochroleuca* and autohydrolysis products

		L. ochroleuca	Solid residue	Liquor
	Moisture (g (100 g)$^{-1}$)	9.20 ± 0.07	3.63 ± 0.08	97.81 ± 0.01
	Ash (g (100 g)$^{-1}$, d.b)	35.01 ± 0.31	17.54 ± 0.07	10.28 ± 0.16
	Protein (g (100 g)$^{-1}$, d.b)	9.21 ± 0.12	15.85 ± 0.31	5.61 ± 0.02
	Sulphates (g (100 g)$^{-1}$, d.b)	2.21 ± 0.10	2.11 ± 0.08	14.54 ± 2.2
Minerals (mg kg^{-1})	Calcium (Ca)	7154	14,393	3441
	Potassium (K)	107,314	44,178	175,156
	Magnesium (Mg)	5032	3134	6310
	Sodium (Na)	29,070	11,309	46,956
	Phosphorus (P)	1872	850	2628
	Iodine (I)	4130	1600	6760
	Zinc (Zn)	n.d.	40	n.d.
Heavy metals (mg kg^{-1})	Arsenic (As)	41.3	30.6	43.2
	Cadmium (Cd)	0.7	1.0	0.7
	Copper (Cu)	< 1	2.7	< 1
	Iron (Fe)	22.1	56	3.5
	Lead (Pb)	< 2.2	< 2	< 2.3
	Mercury (Hg)	n.d.	0.065	n.d.

Data are shown as mean ± standard deviation, in dry basis. All data without standard deviations exhibited standard deviations below 2%

there has been an increasing interest in algae research and food development using seaweeds (e.g. López-López et al. 2011; del Olmo et al. 2018). Probably to its gastronomy tradition of using seaweeds for human consumption, on the 1990s, France sets a recommendation on the maximum heavy metal limits: 3 mg kg^{-1} As, 0.1 mg kg^{-1} Hg, 5 mg kg^{-1} Pb (Benoit 2016; AFSSA 2009; Circuncisão et al. 2018). On a regulatory point of view, the Europe legislation only sets maximum allowed levels of Cd (3 mg kg^{-1}) and Pb (3 mg kg^{-1}) in foodstuffs (Reg. CE 629/2008). This issue is on today's agenda, so in recent years, there has been some efforts made towards a European standardisation on this matter. The European Commission set a recommendation for monitoring the presence of arsenic in food, between 2016 and 2018 to enable an accurate estimation of As exposure (EC 2015b). The FAO/WHO Joint Expert Committee on Food Additives (JECFA) also established for cadmium limits for assumed safe intakes, the provisional tolerable weekly dose (PTWI, μg kg^{-1} of body weight in a week) that an adult man can absorb without health damage. These levels have been adjusted throughout the years, and in 2011 were set in 2.5 μg of cadmium kg^{-1} body weight in order to ensure a high level of protection of consumers (EFSA 2012).

Our results show that of the analysed heavy metals, only arsenic levels may be of concern in *L. ochroleuca* and its AH fractions, but since a speciation of the chemical forms of arsenic was not performed in this study, only the total arsenic content is presented. Although some studies show that heavy metal levels of brown algae can be reduced by application of AH (e.g. Saravana et al. 2016), we did not observed this tendency. Moreover, during seaweed metabolic process, inorganic As can be converted into organic forms, less harmful to human health (Taylor et al. 2017).

As already mentioned, membrane technology was used to obtain purified fractions from the AH liquor. Both AH liquor and the membrane fractions were characterised in terms of antioxidant activity (Table 2). The most relevant feature is that the fractioning of AH liquor resulted in a significant ($p < 0.05$) increase in phlorotannin and sulphate contents that showed an increasing trend with decreasing MWCO although without statistical significance. These values are comparable with those obtained by microwave hydrogravity (MHG) from *L. ochroleuca* (López-Hortas et al. 2018). Concerning the scavenging activity, the highest MWCO tested fraction (> 100 kDa) exhibited the largest values. This behaviour nicely matches those reported for other brown seaweeds (*Sargassum muticum*) processed by membranes (Álvarez-Viñas et al. 2019), although the enhancement found here was much lower than that identified in *S. muticum*. A decreasing trend in this parameter was identified to lower membrane size pore, but not with the phenolic content. Even though, the values obtained here were higher than those obtained for the same alga (*L. ochroleuca*) using other green technology as microwave hydrogravity (López-Hortas et al. 2018), reinforcing the formation of novel bioactive compounds using AH.

Regarding the colour parameters of *L. ochroleuca*, AH products and purified fractions (Table S3), there are noticeable differences between *L. ochroleuca* milled fractions, with smaller particle size fraction being lighter, due mainly to the L^* value, which is directly related to the surface area

Table 2 Antioxidant activity of AH liquor and purified fractions from *Laminaria ochroleuca*

	Phlorotannins (mg PGE g^{-1} extract d.b)	ABTS (mg Trolox g^{-1} extract d.b)	Sulphates (mg g^{-1} extract d.b)
Liquor	4.65 ± 0.10^{b}	7.05 ± 0.01^{b}	145.4 ± 2.2^{b}
R100	5.72 ± 0.03^{a}	7.91 ± 0.01^{a}	417.9 ± 8.7^{a}
R50	6.05 ± 0.32^{a}	5.42 ± 0.01^{c}	103.5 ± 0.8^{c}
R30	6.18 ± 0.53^{a}	1.81 ± 0.01^{d}	88.4 ± 5.0^{d}
P30	5.62 ± 0.04^{a}	1.55 ± 0.01^{e}	73.3 ± 3.1^{e}

Data are presented as mean ± standard deviation. Different superscript letters in a column show significantly different data values at $p \leq 0.05$ level

differences. The ΔE^* between purified fractions and AH liquor ranges from 3.4 to 6.4, indicating that, apart from R100, the mentioned colour differences are detected by normal human vision (Castellar et al. 2006). The brownish colour of AH liquor is probably due to the formation of new compounds by Maillard and caramelization reactions, which could be favoured by extraction conditions, namely high temperature. These new compounds can possess antioxidant activity as reported by previous studies with plants (e.g. *Rosmarinus officinalis* L., *Thymus vulgaris*), microalgae (*Chlorella vulgaris*), and seaweeds (e.g. *Porphyra* spp., *Undaria pinnatifida, Sargassum muticum, Saccharina japonica*) (Plaza et al. 2010; Saravana et al. 2016).

Molecular mass distribution

From the HPSEC spectra of *L. ochroleuca* AH liquor and their corresponding fractions (Figure S1), it is clearly observed that all systems exhibited high molecular weight (> 80 kDa). Liquor, > 100 kDa and 50–100 kDa featured a similar profile. In the latter two fractions, the use of the membranes led to concentrate polymers with higher molecular weight. In the membranes with the lowest pore size, it was observed depolymerization of the polysaccharides presents in the liquor, with peaks close to 80 kDa. These two trends are consistent with phytochemical results.

Oligosaccharides

The maximum fucose oligosaccharide content (9.84 g (100 g)$^{-1}$ extract) was found in the > 100 kDa fraction (Fig. 2) matching with the maximum sulphate content (417.9 mg g^{-1} extract) (Table 2). The fucose content was followed by glucose, galactose, mannose, and xylose oligosaccharides in the higher membranes. In contrast, the lower cut-off membranes exhibited a different trend. These results are consistent with the phytochemical results previously explained.

In a recent study, Flórez-Fernandez et al. (2019) performed autohydrolysis at temperatures between 120–220 °C with *L. ochroleuca*. These authors found that the oligosaccharides content continued to increase up to 180 °C, when they start to decrease, probably due to caramelization and Maillard reactions (Saravana et al. 2016).

Characterisation of purée-like mixtures

Texture measurements

The texture profile of the purée-like mixtures and the commercial samples (pasta filling and elderly food) are depicted in Fig. 3 (texture parameters extracted, Table S2). The texture profiles of the alga systems vary according to the UAE treatment (Fig. 3a). As already mentioned, UAE results in the release of bioactive compounds into the medium. This fact translates in a significant adhesiveness gain for the system from − 0.31 to − 0.92 N.s. This could be associated with a release of phospholipids or glycoproteins from the algae cell wall. However, the same treatment combined with temperature (A_US80) originated a product significantly less firm and adhesive. This thermal behaviour is consistent with that found for other food matrices, where denaturation and structural changes of proteins were suggested as responsible for the adhesiveness decrease (Pérez-Santaescolástica et al. 2018). These authors also indicated that the combined thermal and ultrasonic cavitation effect could cause loosening of the molecular structure and reduction of molecular nodes.

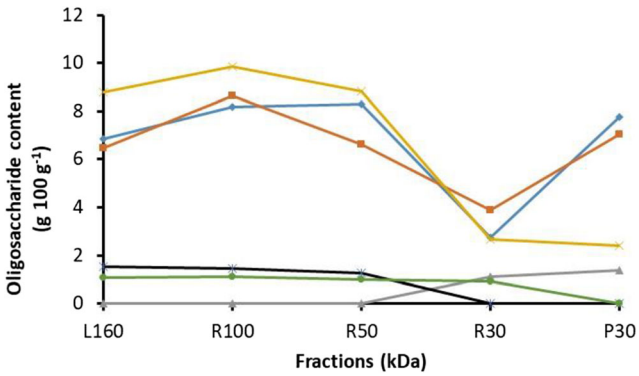

Fig. 2 Oligosaccharide and sulphate contents of liquid extract obtained from autohydrolysis (L160) and the corresponding fractions. Symbols: O-Glucose; O-Ga+Xy+Mn; O-Rhamnose; O-Fucose; Formic Groups; Acetyl Groups

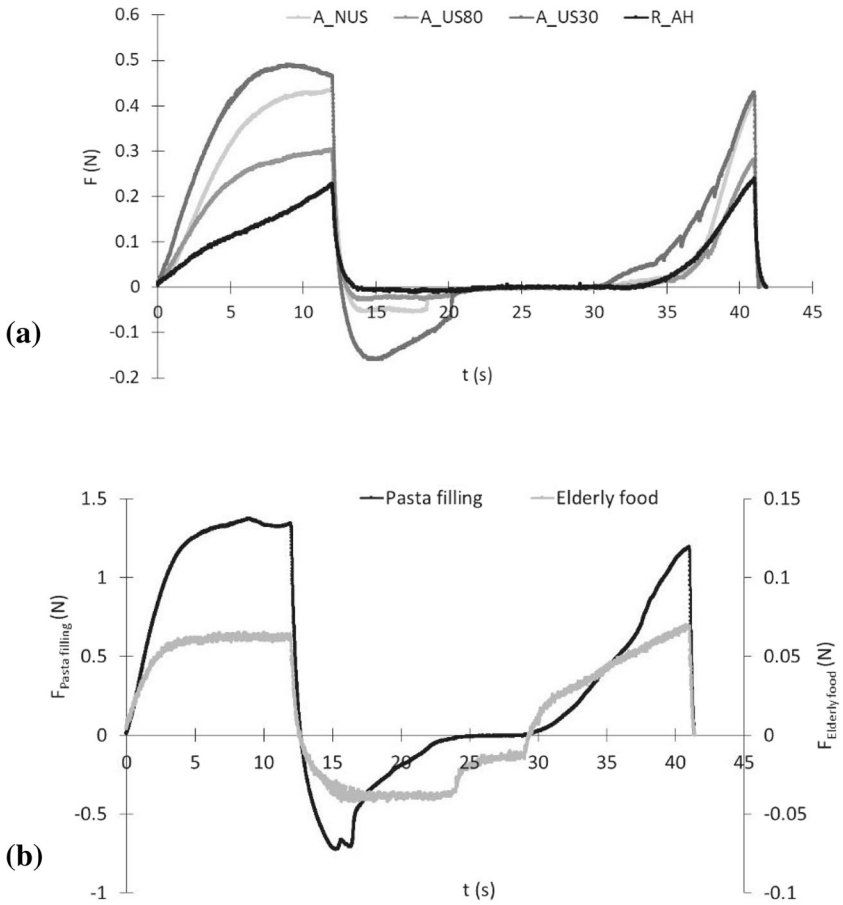

Fig. 3 Texture profile analysis (TPA) for the different tested systems **a** prepared with *L. ochroleuca* brown seaweed (i.e. A_NUS, A_US30, A_US80, R_AH) as well as **b** those commercially available (i.e. Pasta filling and Elderly food). A_NUS (Alga 20%, 90 °C/30 min), A_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min)

Comparing the texture profile of the purée-like systems developed with the commercial food products selected (Fig. 3), A_US30 is the system that most resembles the pasta filling. Although some differences are observed, especially in their firmness and adhesiveness values (Table S2), it should be pointed out that the systems developed are not finished products, but systems consisting of only alga/residue and water. According to a Finnish Project Report on Food for Seniors (Heiniö et al. 2014), the textures between soft and semi-hard were the most preferred by senior consumers, indicating that some but not too big textural challenges are appreciated. Furthermore, they prefer salty dishes, which can point to the development of the type of products suggested in the present study.

The EC regulation is absent about the use of AH solid residue for food applications. However, the sensorial perception, namely the characteristic aroma, could be an issue. We suggest the use of this raw material in non-food applications, for instance, cosmetic or personal care products.

Thermorheological measurements

At the beginning of the heating process, all samples showed a predominantly viscous behaviour, with G″ being higher than G′ (Fig, 4a). Although practically no differences related to the sample structure are observed, R_AH presented a slightly different heating profile than the alga samples. At around 35 °C, there is a crossing of the moduli for all samples that suggests sample structuration due to water uptake from the protein, fibre (*Laminaria japonica*: 50.7 g (100 g)$^{-1}$) or sugars present in the sample (Moreira et al. 2014).

During the cooling process (Fig. 4b), all samples showed a linear increase of both viscoelastic moduli, revealing an increase in structuring as the temperature lowers. The three alga samples showed a similar profile, and different from R_AH, where the rate of structuring is not so pronounced ($G'_{90°C} \rightarrow G'_{5°C}$: 200–600 Pa), whereas for the other samples G′ increases more than 60 times. Fucoidan, the main polysaccharide of *L. ochroleuca*, is very soluble once extracted and the solubility is related to the level of branching, depending on the content of sulphate groups (Cunha and Grenha 2016). During AH processing, algae polysaccharides, namely fucoidan, laminarin, alginate, and its fractions, are released into the liquid extract (Wan et al. 2015), what explains why in R_AH the degree of structuring is lower than in the alga samples, where these polysaccharides are still present. Although fucoidan does not develop highly viscous solutions

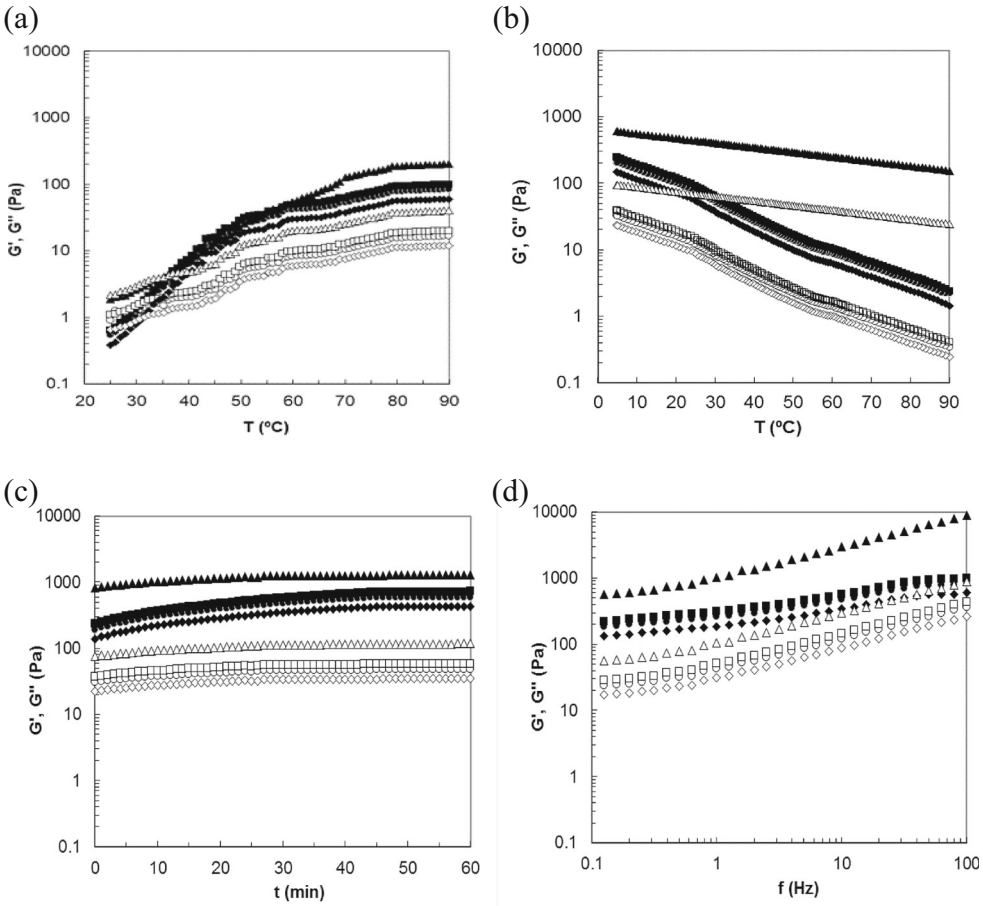

Fig. 4 Thermorheology of tested systems prepared with *L. ochroleuca* brown seaweed: **a** temperature profiles on heating (25 to 90 °C at 2 °C min^{-1}) and **b** cooling (90 to 5 °C at 1 °C min^{-1}), **c** time sweep (5 °C), **d** frequency sweep (25 °C). Symbols: circles (A_NUS), diamonds (A_US80), squares (A_US30), triangles (R_AH), closed (storage modulus, G′), open (loss modulus, G″), A_NUS (Alga 20%, 90 °C/30 min), A_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min)

(Rupérez 2002), in fact, it produces aqueous solutions of low apparent viscosity with shear-thinning flow behaviour; alginate forms very viscous solutions (Flórez-Fernández et al. 2019) that can be responsible for the differences obtained between samples.

Regarding the maturation kinetics (Fig. 4c) of the systems, R_AH is almost instantaneously matured after the cooling process, whereas the alga samples take about 45 min to attain full maturation. At this point, it is clear that A_US80 shows a slightly weak structure than the other samples, indicating that the temperature in the UAE (and not the UAE) is responsible for the weakening of the structure of this sample. At 80 °C, the proteins in this sample are already fully denatured and not able to form entanglements that result in structural reinforcement.

As depicted in the mechanical spectra (Fig. 4d), these material structure is consistent with pastes, i.e. purée-like systems, what can be explained by the swelling (10.20 ± 0.37 mL g^{-1}, d.b), and water absorption capacity (8.93 ± 0.52 g g^{-1}, d.b) of *Laminaria* (Gómez-Ordóñez et al. 2010). These values are similar to those from *Psyllium* husk, well-known for its water uptake capacity (Raymundo et al. 2014).

Apart from processing conditions in texture and rheology measurements, the results of the mechanical spectra are in agreement with the ones from texture analysis (Fig. 3a). Although R_AH presents higher viscoelastic modulli in the mechanical spectrum, it is highly frequency dependent (especially at higher frequency values), indicating a less structured material. This reflects the more cohesive and less firm structure of R_AH when compared with alga samples (Table S1).

Colour and syneresis

As expected, in terms of colour (Table S3), R_AH is distinctively different from the other systems. It presents a darker brownish colour, whereas the algae systems are greener. The ΔE^* between the other alga mixtures ranges from 1.26 to 2.45, below the threshold of normal human vision (Castellar et al. 2006). Although none of the samples presented syneresis, R_AH showed a slight tendency for syneresis with time (not measured). Both of these parameters could be of interest in future applications.

Bioactive compounds measurements

Purée-like mixtures exhibited a wide range of sulphate content (A_NUS: 1.93 ± 0.6 g (100 g)$^{-1}$, d.b.) increasing with UAE (A_US30: 4.51 ± 1.3 g (100 g)$^{-1}$, d.b.) and AH (R_AH: 13.43 ± 4.7 g (100 g)$^{-1}$, d.b.) processing. Note that the sulphate

content of A_US80 was 2.46 ± 0.60 g (100 g)$^{-1}$, d.b., lower than all alga pretreated systems. This suggests that some interferences could take place with biopolymers as gelling starch fractions or denatured proteins at 80 °C. In general, the sulphate content of the raw material (Table 1), when compared with the content developed in the purée-like mixtures, exhibited a huge increase. This is probably due to both processing methodologies that favour the solubilisation of compounds and their release into the medium, behaviour consistent with the observed for other brown seaweeds (Flórez-Fernández et al. 2017).

Concerning phlorotannin content, the purée-like mixture pretreatments could allow the formulation of final products with the seaweed or solid residue from AH with values in the same order of magnitude as those obtained in AH liquor (4.65 mg g^{-1} extract), reinforcing the functional potential of the developed matrices. However, the antioxidant activity of these systems could be increased by replacing the water with the AH liquor, since subcritical water extraction allows the formation of new compounds with antioxidant capacity, as already mentioned and reported by several authors (Plaza et al. 2010; González-López et al. 2012). For this purpose, Shibata et al. (2008) suggest that a complex of crude phlorotannins and soybean protein may be useful as a new functional foodstuff with antioxidant and anti-inflammatory activity. To our knowledge, there are no studies with the whole alga processed in the way proposed in this study. These purée-like mixtures could be suited for food applications as a source of minerals or may serve as a food supplement to help meet the recommended daily intakes of some minerals and trace elements (Rupérez 2002). Ageing is also associated with the insufficient intake of micronutrients, which leads to global nutritional deficiency. In this way, the intake of foods rich in vitamins and minerals is recommended for this age group population. Overall, this work represents a preliminary study where proposed purée-like mixtures could be an attractive alternative for specific target population groups. Anyway, before developing any final product formulation, the heavy metal content as arsenic present in the algae formulation should be further studied and surveyed. Even though, previous works (e.g. García-Sartal et al. 2013) indicated that heavy metals present in *L. ochroleuca* exhibited a negligible bioavailability after cooking.

Conclusions

This approach based on the integral use of *L. ochroleuca* is an alternative to the current production process of chemicals from seaweed focused on a single product (e.g. alginate) and to the more recent cascade biorefinery. Besides, the incorporation of whole algae promotes the simultaneous presence of different components (dietary fibre, protein, minerals, vitamins, carotenoids, phlorotannins, etc.), with both health-beneficial effects and technological advantages. Future studies are being performed to evaluate the effect of the incorporation of the algae mixtures with gelling agents to expand the spectra of applications.

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References

AFSSA (2009) Opinion of the French Food Safety Agency on the recommended maximum inorganic arsenic content of laminaria and consumption of these seaweeds in light of their high iodine content. AFSSA Request no. 2007-SA-0007

Álvarez-Viñas M, Flórez-Fernández N, González-Muñoz MJ, Domínguez H (2019) Influence of molecular weight on the properties of *Sargassum muticum* fucoidan. Algal Res 38:101393

Benoit M (2016) Seaweed Standards for food and cosmetics. Seagriculture – 5th International Seaweed Conference. Aveiro, Portugal. https://seagriculture.eu/wp-content/upload_folders/seagriculture.eu/2016/10/2.3-Maud-Benoit-Seaweed-standards-for-food-and-cosmetics.pdf

Castellar MR, Obón JM, Fernández-López JA (2006) The isolation and properties of a concentrated red-purple betacyanin food colorant from *Opuntia stricta* fruits. J Sci Food Agric 86:122–128

Castro-Puyana M, Herrero M, Mendiola JÁ, Ibáñez E (2013) Subcritical water extraction of bioactive components from algae. In: Domínguez H (ed) Functional ingredients from algae for foods and nutraceuticals. Woodhead Publishing, Cambridge, pp 534–560

Černá M (2011) Seaweed proteins and amino acids as nutraceuticals. In: Kim S-K (ed) Advances in food and nutrition research. Elsevier, Amsterdam, pp 297–312

Circuncisão AR, Catarino MD, Cardoso SM, Silva AMS (2018) Minerals from macroalgae origin: health benefits and risks for consumer. Mar Drugs 16:400

Conde E, Balboa EM, Parada M (2013) Algal proteins, peptides and ainoacids. In: Domínguez H (ed) Functional ingredients from algae for foods and nutraceuticals. Woodhead Publishing, Cambridge, pp 135–180

Cunha L, Grenha A (2016) Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. Mar Drugs 14:42

del Olmo A, Picon A, Nuñez M (2018) Cheese supplementation with five species of edible seaweeds: effect on microbiota, antioxidant activity, colour, texture and sensory characteristics. Int Dairy J 84:36–45

Dodgson KS (1961) Determination of inorganic sulphate in studies on the enzymatic and non-enzymatic hydrolysis of carbohydrate and other sulphate esters. Biochem J 78:312–319

EFSA (2012) Cadmium dietary exposure in the European population. Scientific Report of EFSA. EFSA J 10:2551

European Commission (2008) Commission Regulation (EC) N° 629/2008 amending Regulation (EC) N° 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Brussels, Official Journal of the European Union 176:6–9

European Commission (2015a) Closing the loop - an EU action plan for the circular economy. In: Brussels, COM(2015)614. Journal of the European Union, Official

European Commission (2015b) Commission Recommendation 2015/1381 on the monitoring of arsenic in food. Brussels, Official Journal of the European Union 213:9–10

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-Pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (*Ochrophyta*) as part of the flexible response to highly variable marine environments. J Appl Phycol 28:3689–3696

Fernández-Fernández AM, Moreda-Piñeiro A, Bermejo-Barrera P (2007) On-line preconcentration cold vapour atomic absorption spectrometry for the determination of trace mercury in edible seaweeds. J Anal At Spectrom 22:573–577

Flórez N, Gonzalez-Muñoz MJ, Ribeiro D, Fernandes E, Dominguez H, Freitas M (2017) Algae polysaccharides' chemical characterization and their role in the inflammatory process. Curr Med Chem 24:149–175

Flórez-Fernández N, López-García M, González-Muñoz MJ, López-Vilariño JM, Domínguez H (2017) Ultrasound-assisted extraction of fucoidan from *Sargassum muticum*. J Appl Phycol 29:1553–1561

Flórez-Fernández N, Torres MD, González-Muñoz MJ, Domínguez H (2019) Recovery of bioactive and gelling extracts from edible brown seaweed *Laminaria ochroleuca* by non-isothermal autohydrolysis. Food Chem 277:353–361

García-Sartal C, Barciela-Alonso MC, Moreda-Piñeiro A, Bermejo-Barrera P (2013) Study of cooking on the bioavailability of As, Co, Cr, Cu, Fe, Ni, Se and Zn from edible seaweed. Microchem J 108:92–99

Gómez-Ordóñez E, Jiménez-Escrig A, Rupérez P (2010) Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. Food Res Int 43:2289–2294

González-López N, Moure A, Domínguez H (2012) Hydrothermal fractionation of *Sargassum muticum* biomass. J Appl Phycol 24:1569–1578

Heiniö R-L, Pentikäinen S, Rusko E, Peura-Kapanen L (2014) Food for seniors – final report. National Consumer Research Centre, VTT Technology 202. ISBN 978-951-38-8200-6. http://www.vtt.fi/publications/index.jsp. Accessed 27 December 2018.

Kadam SU, O'Donnell CP, Rai DK, Hossain MB, Burgess CM, Walsh D, Tiwari BK (2015a) Laminarin from Irish brown seaweeds *Ascophyllum nodosum* and *Laminaria hyperborea*: ultrasound assisted extraction, characterization and bioactivity. Mar Drugs 13:4270–4280

Kadam SU, Tiwari BK, Smyth TJ, O'Donnell CP (2015b) Optimization of ultrasound assisted extraction of bioactive components from brown seaweed *Ascophyllum nodosum* using response surface methodology. Ultrason Sonochem 23:308–316

Kim S-K, Bhatnagar I (2011) Physical, chemical, and biological properties of wonder kelp—*Laminaria*. In: Kim S-K (ed) Advances in food and nutrition research. Elsevier, Amsterdam, pp 85–96

Koivikko R, Loponen J, Honkanen T, Jormalainen V (2005) Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. J Chem Ecol 31:195–212

Lodeiro P, Cordero B, Barriada JL, Herrero R, Sastre de Vicente ME (2005) Biosorption of cadmium by biomass of brown marine macroalgae. Bioresour Technol 96:1796–1803

López-Hortas L, Gannon L, Moreira R, Chenlo F, Domínguez H, Torres MD (2018) Microwave hydrodiffusion and gravity (MHG) processing of *Laminaria ochroleuca* brown seaweed. J Clean Prod 197:1108–1116

López-López I, Cofrades S, Cañeque V, Díaz MT, López O, Jiménez-Colmenero F (2011) Effect of cooking on the chemical composition of low-salt, low-fat Wakame/olive oil added beef patties with special reference to fatty acid content. Meat Sci 89:27–34

Lourenço SO, Barbarino E, De-Paula JC, Pereira LODS, Lanfer Marquez UM (2002) Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol Res 50:233–241

Martínez-Hernández GB, Castillejo N, Carrión-Monteagudo MM, Artés F, Artés-Hernández F (2017) Nutritional and bioactive compounds of commercialized algae powders used as food supplements. Food Sci Technol Int 24:172–182

Moreira R, Chenlo F, Torres MD, Rama B (2014) Fine particle size chestnut flour doughs rheology: influence of additives. J Food Eng 120:94–99

Overend R, Chornet E (1987) Fractionation of lignocellulosics by steam-aqueous pretreatments. Phil Trans R Soc Lond A 321:523–536

Pérez-Santaescolástica C, Carballo J, Fulladosa E, Garcia-Perez JV, Benedito J, Lorenzo JM (2018) Application of temperature and ultrasound as corrective measures to decrease the adhesiveness in dry-cured ham. Influence on free amino acid and volatile compound profile. Food Res Int 114:140–150

Plaza M, Amigo-Benavent M, del Castillo MD, Ibáñez E, Herrero M (2010) Facts about the formation of new antioxidants in natural samples after subcritical water extraction. Food Res Int 43:2341–2348

Raymundo A, Fradinho P, Nunes MC (2014) Effect of *Psyllium* fibre content on the textural and rheological characteristics of biscuit and biscuit dough. Bioact Carbohydr Dietary Fibre 3:96–105

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

Rupérez P (2002) Mineral content of edible marine seaweeds. Food Chem 79:23–26

Sánchez-Machado DI, López-Cervantes J, López-Hernández J, Paseiro-Losada P (2004) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chem 85:439–444

Saravana PS, Choi JH, Park YB, Woo HC, Chun BS (2016) Evaluation of the chemical composition of brown seaweed (*Saccharina japonica*) hydrolysate by pressurized hot water extraction. Algal Res 13:246–254

Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H, Hama Y (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. J Appl Phycol 20:705–711

Taylor VF, Li Z, Sayarath V, Palys TJ, Morse KR, Scholz-Bright RA, Karagas MR (2017) Distinct arsenic metabolites following seaweed consumption in humans. Sci Rep 7:3920

Wan P, Yang X, Cai B, Chen H, Sun H, Chen D, Pan J (2015) Ultrasonic extraction of polysaccharides from *Laminaria japonica* and their antioxidative and glycosidase inhibitory activities. J Ocean Univ China 14:651–662

WHO (2015). World Report on Ageing and Health. ISBN 9789241565042

Supplementary material

Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties

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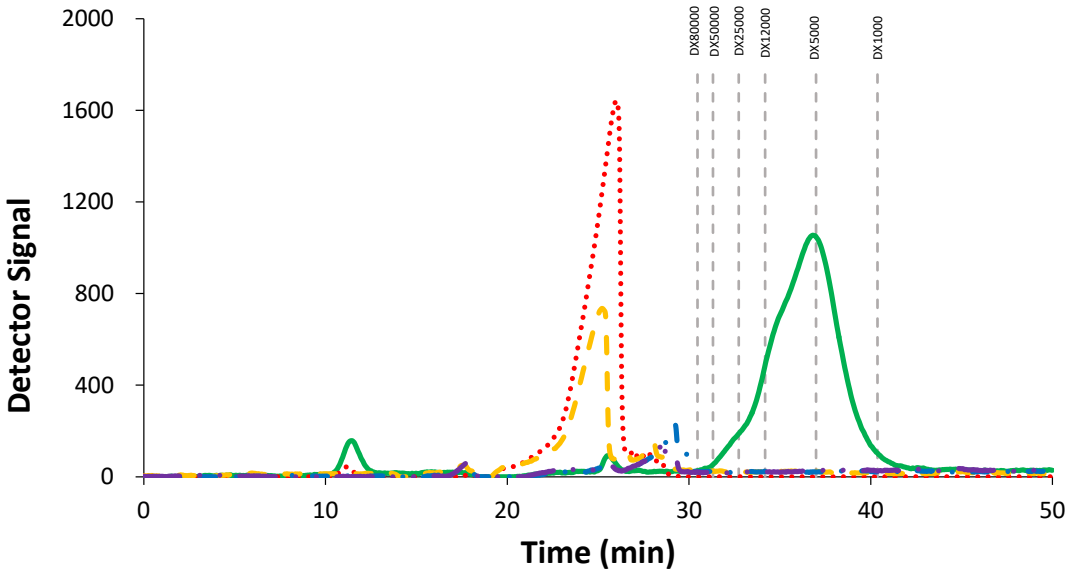

Fig. S1 HPSEC spectrum of liquid fractions from *Laminaria ochroleuca.*

Symbols: L160; R100; R50; R30; P30

Table S1 Colour parameters of *L. ochroleuca*, AH products and purified fractions

		L*	**a***	**b***	**ΔE***
L. ochroleuca	<0.25 mm	56.36 ± 0.57	-2.48 ± 0.04	14.49 ± 0.18	17.1
	0.25-2 mm	40.67 ± 0.62	-1.32 ± 0.15	7.80 ± 0.59	
Autohydrolysis fractions	Solid residue	27.56 ± 0.39	0.28 ± 0.08	6.73 ± 0.14	-
	Liquor	31.42 ± 1.74	1.45 ± 0.26	2.88 ± 1.60	-
Purified fractions	R100 kDa	33.92 ± 0.68^{a}	1.24 ± 0.07^{x}	0.57 ± 0.21^{m}	3.4
	R50 kDa	34.70 ± 1.79^{a}	1.39 ± 0.16^{x,y}	-1.04 ± 0.64^{n}	5.1
	R30 kDa	35.66 ± 1.33^{a}	1.50 ± 0.10^{y}	-1.93 ± 0.20^{o}	6.4
	P30 kDa	33.90 ± 0.91^{a}	1.45 ± 0.07^{y}	-1.67 ± 0.12^{o}	5.2

Data are presented as mean ± standard deviation. Within the same group sample (Purified fractions) different superscript letters in a column show significantly different data values at $p \leq 0.05$ level. ΔE* of purified fractions was determined in relation to liquor.

Table S2 Texture parameters for purée-like mixtures and commercial references

Sample	**Firmness (N)**	**Adhesiveness (N·s)**	**Cohesiveness**
A_NUS	0.44 ± 0.03^{b}	- 0.31 ± 0.04^{c}	0.30 ± 0.04^{c,d}
A_US80	0.34 ± 0.05^{c}	- 0.18 ± 0.03^{d}	0.28 ± 0.02^{c}
A_US30	0.51 ± 0.08^{b}	- 0.92 ± 0.13^{b}	0.35 ± 0.05^{b,c}
R_AH	0.24 ± 0.03^{d}	- 0.06 ± 0.02^{e}	0.48 ± 0.04^{b}
 **Elderly food**	0.07 ± 0.01^{e}	- 0.46 ± 0.11^{c}	0.73 ± 0.12^{a}
 **Pasta filling**	1.35 ± 0.16^{a}	- 3.66 ± 0.34^{a}	0.44 ± 0.06^{b}

Data are presented as mean ± standard deviation. Data values in a column with different superscript letters are significantly different at the $p \leq 0.05$ level. A_NUS (Alga 20 g/100g, 90 °C/30 min), A_US30

(Alga 20 g/100g, 90 °C/30 min, UAE 30 °C/30 min) and A_US80 (Alga 20 g/100g, 90 °C/30 min, UAE 80 °C/30 min)

Table S3 – Colour parameters of purée-like mixtures

	A_NUS	**A_US80**	**A_US30**	**R_AH**
L*	24.91 ± 1.51^{x}	24.15 ± 1.69^{x}	24.60 ± 1.06^{x}	21.49 ± 0.69^{y}
a*	-0.70 ± 0.11^{a}	-0.61 ± 0.08^{a}	-0.65 ± 0.13^{a}	1.05 ± 0.21^{b}
b*	8.28 ± 0.60^{o}	7.27 ± 0.41^{o}	9.68 ± 1.05^{n}	5.05 ± 0.34^{m}

Data are presented as mean ± standard deviation. Different superscript letters in a row show significantly different data values at $p \leq 0.05$ level. A_NUS (Alga 20 g/100g, 90 °C/30 min), A_US30 (Alga 20 g/100g, 90 °C/30 min, UAE 30 °C/30 min) and A_US80 (Alga 20 g/100g, 90 °C/30 min, UAE 80 °C/30 min)

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Psyllium and *Laminaria* Partnership—An Overview of Possible Food Gel Applications

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Featured Application: *Laminaria-psyllium* gels with distinct texture and rheological features, designed for a wide range of food applications.

Abstract: Seaweeds are a novel source of important nutritional compounds with interesting biological activities that could be processed into added-value products. In this study, two previously developed products obtained by *Laminaria ochroleuca* processing (liquid extract and a purée-like mixture) were processed with *Psyllium* gel to develop functional hydrogels. The optimization of the formulation and the characterization of the *Laminaria-Psyllium* gels in terms of their mechanical features have allowed the proposal of potential food applications. A beneficial interaction was found between *Laminaria* and *Psyllium* in terms of the reinforcement of texture and rheological properties. The obtained outcomes could provide new healthy gelling formulations with attractive properties to alleviate the growing market demand of eco-novel food matrices.

Keywords: kombu; edible brown seaweed; gels; *Psyllium*; *Laminaria ochroleuca*; autohydrolysis; mechanical properties

1. Introduction

Laminaria sp. are industrially used for alginate extraction (17.1–32% *w/w*, dry basis) [1], a hydrocolloid with unique gelling abilities at low temperature and good heat stability, widely used as a thickener, stabilizer, and restructuring agent in the food, cosmetic, pharmaceutical, biomedical, and textile industries [2,3].

Mainly valued for alginate extraction, this natural resource presents other interesting compounds for human nutrition such as fatty acids, proteins, minerals [4], vitamins (A, C, D, B group, E, K, PP) [1], pigments such as carotenoids and polyphenols with proved antioxidant, hypoglycemic, antitumoral, and antimicrobial activities [5,6]. Due to their high content in biologically active compounds, seaweeds, and especially *Phaeophyceae* (brown algae), have great potential to act as a functional food and as a food ingredient [7,8].

Psyllium (*Plantago ovata*) is an annual plant found mainly in India and Pakistan, but also on Madeira Island (Portugal) due to its subtropical climate. Used in traditional medicine for centuries, *Psyllium* husk has gained more attention by the scientific community since its health allegation was approved by the Food and Drug Administration in 2012 [9] regarding its benefits in reducing the risk

of coronary heart disease. These features come from its high soluble fiber composition, which has the capacity of absorbing up to 15 g of water per g of *Psyllium* [10].

Psyllium husk is composed of 85% arabinoxylan, a neutral highly branched polysaccharide with about 35% of non-reducing terminal residues and a 15% non-polysaccharide fraction [11]. It has applications as an edible coating [12] and drug delivery [13].

Innovation in food and feed production is a reality and a tendency for the upcoming years, whether this is the use of poorly exploited raw materials, the reformulation of foods based on green and sustainable technologies, or "back to tradition". Many of the food and feed industries' products are gels (e.g., yoghurt, puddings, confectionery products, pasta, pet food, among others) or present gelling agents in their formulation (cream cheese, sauces, ...), making gel-systems a growing market.

Among the edible biopolymers, the use of plant seed mucilages namely chia and flax seed [14,15] are one of today's trends. Aside from the sustainability issue, these ingredients also have health benefits such as the regulation of colonic microbiota, reduction of hyperlipidemia, anti-inflammatory effect, control of glycemic response, and control of satiation [16]. Due to their technological properties, these biopolymers are often used in the food industry as texturizing agents.

To our knowledge, the gel forming ability of the combination of *L. ochroleuca-Psyllium* has not been previously investigated. Therefore, the interaction between *Psyllium* husk gel and the edible brown seaweed *Laminaria ochroleuca*, either in its liquid fraction or its purée-like mixtures, with *Psyllium* will be studied, taking advantage of both these poorly exploited natural resources. The aim of the present work is to lay the foundation for a systematic textural and rheological description of *Laminaria-Psyllium* gels, focusing on their mechanical features intended for future food applications.

2. Materials and Methods

2.1. Raw Materials

Dehydrated *Laminaria ochroleuca* (Algas Atlánticas Algamar, S.L., Pontevedra, Spain) was milled and sieved to two different particle size powders (0.25–2 mm and <0.25 mm diameters). *Psyllium* husk of Indian origin (Solgar, lot 107028-01, Leonia, NJ, USA) was purchased at the local market, milled (Pulverisette 14 Premium, Fritsch, Idar-Oberstein, Germany) and sieved to 160–315 µm.

For comparison purposes, commercial references were used, namely baby food (purée), guacamole (purée), fruit jam (gel), jelly gum (rubbery solid), pâté (liver paste), and pet food (moistened feed, pâté like).

2.2. Formulations Development

2.2.1. *Laminaria ochroleuca* Sample Production

Focusing on the full valorization of *L. ochroleuca*, the 2 mm fraction of the alga was subjected to autohydrolysis (AH) in a pressurized reactor (Parr Instruments series 4848, Moline, IL, USA) [14] and the liquid extract (liquor) obtained was stored at −18 °C until further use. When using the <0.25 mm alga fraction, purée-like mixtures were prepared following the procedure already described by the authors [17], with (LoUS) and without ultrasonic treatment (Lo). The alga purées were matured for 24 h at 4 °C before preparing the *Laminaria-Psyllium* gels.

2.2.2. Preparation of the *Laminaria-Psyllium* Gels

The control sample (Psy) was prepared by adding 4% (*w*/*w*, d.b) *Psyllium* husk (160–315 µm range of particle size) to distilled water at 40 °C under mechanical stirring (10 min, 200 rpm) [18]. Another *Psyllium* gel was prepared in a similar manner as the control by using the *Laminaria* autohydrolysis liquid extract (PsyL).

Laminaria-Psyllium gels were prepared by mixing (Eurostar Power-b, Ika-Werke, Staufen im Breisgau, Germany) for 5 min/200 rpm at room temperature, the control sample and the alga purée

mixture at the ratios 25:75, 50:50, and 75:25 in order to obtain visually different structures with potential different applications. Two batches of 160 g of each gel (*Laminaria-Psyllium*: 25:75, 50:50, 75:25) were prepared, poured into sealed plastic containers (Ø = 10 cm) and stored at 4 °C for 24 h to maturate. The schematic procedure of the sample preparation and analysis is presented in Figure 1.

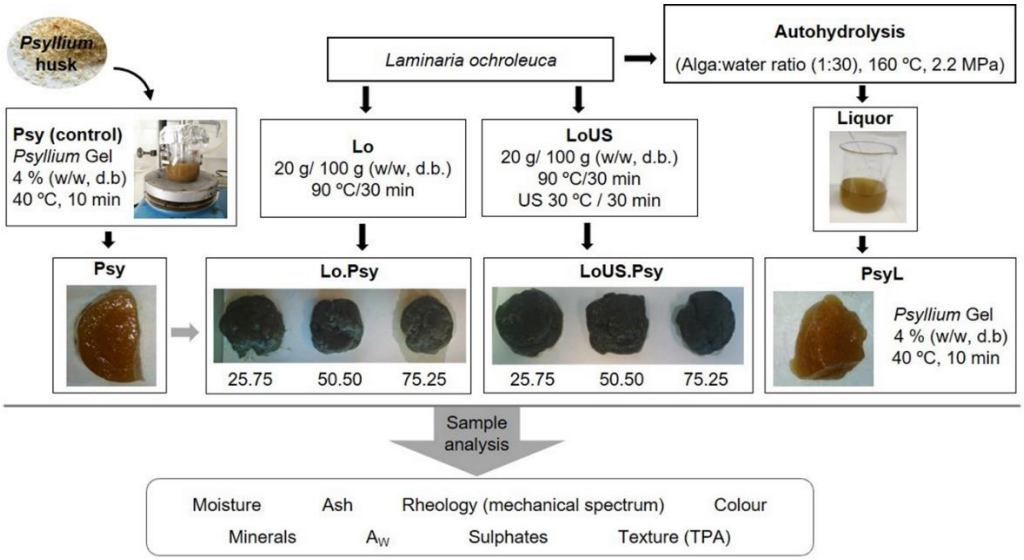


Figure 1. General schematic procedure of the gel preparation and analysis (Lo—*Laminaria ochroleuca*; Psy—*Psyllium* gel prepared with water (control); US—ultrasound treatment; PsyL—*Psyllium* gel prepared with autohydrolysis liquor).

2.3. Physicochemical Measurements

Moisture and ash contents of the raw materials and gels were determined by gravimetric methods by placing the samples in an oven (105 ± 2 °C) until constant weight, or in the furnace (575 °C, 6 h) respectively.

Sulfate content of the developed gel samples and *Psyllium* husk was obtained by the ionic chromatography method (mobile phase: 3.2 mM sodium carbonate/1 mM sodium bicarbonate at 0.70 mL/min) as previously reported [19].

Water activity (a_w) measurements of all gel samples were performed using a LabMaster-aw (Novasina, Pfäffikon, Switzerland) in triplicate.

Mineral content (Ca, K, Na, Fe) of *Psyllium* husk was analyzed by inductively coupled plasma optical emission spectrometry (Optima 4300 DV, Perkin Elmer, Waltham, MA, USA) after microwave-assisted (Savillex, Eden Prairie, MN, USA) acid digestion (80 °C, 6 h).

2.4. Color Measurements

The color evaluation was performed using a CR-400 colorimeter (Minolta, Tokyo, Japan) with standard illuminant C. Tristimulus color coordinates (CIELAB system) were used to measure the degree of lightness (L*), which ranged from 0 (black) to 100 (white), redness (a*) ranging from −60 (green) to +60 (red), and yellowness (b*) ranging from blue (−60) to yellow (+60). Total color difference (ΔE*) between the samples and the control was calculated according to Equation (1).

$$\Delta E^* = (\Delta L^* + \Delta a^* + \Delta b^*)^{1/2} \quad (1)$$

For calibration purposes, a white standard was used (L* = 97.21; a* = 0.14; b* = 1.99). Measurements were conducted at 20 ± 1 °C and replicated at least five times.

2.5. Dynamic Viscoelasticity

Dynamic oscillatory rheology measurements were used to monitor the viscoelastic characteristics of the gels. Small amplitude oscillatory shear measurements were conducted in triplicate in a controlled stress rheometer (RheoStress 600, Haake, Vreden, Germany) using serrated parallel plate geometry (diameter 35 mm) and a 1.5 mm gap. Surface geometry was covered with paraffin oil to prevent moisture loss. Samples were rested in the rheometer device for 5 min (20 °C) before rheological testing to temperature equilibration. Initially, stress sweep tests were run at 1 Hz, with the shear stress of the input signal varying from 0.1 to 100 Pa to find the linear viscoelastic region. The mechanical spectra of all samples were assessed through frequency sweep tests performed from 0.1 to 10 Hz (20 °C, 10 Pa) within the linear viscoelastic region previously defined. Experimental storage (G′) and loss (G″) moduli (Pa) data versus frequency (f, Hz) were fitted using well-known power models reported elsewhere [20], where α′, α″, b′, and b″ are the corresponding fitting parameters (Equations (2) and (3)).

$$G'(f) = \alpha' f^{b'} \quad (2)$$

$$G''(f) = \alpha'' f^{b''} \quad (3)$$

2.6. Texture Profile Analysis (TPA)

Texture profile analysis of all developed gels was performed in a TA-XT2 texturometer (Stable Microsystems, Godalming, UK) with a P0.25S plunger that penetrated 15 mm into the sample at 1 mm/s. From the force vs. time texturogram, three parameters were selected to characterize the materials: firmness, as the maximum rupture force (N); adhesiveness, represented by the negative area of the graph, that translates the recessive force of the probe (N·s); and cohesiveness corresponding to the ratio of the material's response to a second deformation. At least five measurements were made in each sample. Commercial food and feed products were also measured for comparative purposes.

2.7. Statistical Analysis

Statistical analysis of the experimental data was performed using RStudio (Version 1.2.1335—© 2009–2019 RStudio, Inc., Boston, MA, USA), using variance analysis (one-way ANOVA), and the Tukey test, *Post Hoc* comparison at a significance level of 95% ($p < 0.05$). A Pearson correlation analysis was also conducted ($p < 0.05$) to determine the relationships between the color, texture parameters, and the amount of alga of the samples. The curve fitting of the rheological data was performed in Excel (version 365, Microsoft). All results are presented as the mean ± standard deviation (s.d.).

3. Results and Discussion

3.1. Physicochemical Characterization of Samples

Psyllium gel prepared with autohydrolysis (AH) liquor (PsyL) was visually more fluid than the *Psyllium* gel prepared with water (control). This is confirmed by texture and rheology measurements, so this issue will be discussed in Sections 3.3 and 3.4.

As seen in Figure 1, the *Laminaria-Psyllium* 75.25 gel samples presented syneresis (water in the filter paper, not measured) more evident in the sample not submitted to ultrasonic treatment. According to the previous study in which the purée-like mixtures were optimized [17], the authors did not find syneresis in the systems. This could be due to the alga high sodium content that bonds to *Psyllium*, and at the 25% ratio was not able to retain all the water in the system, causing the *Laminaria-Psyllium* 75.25 gels to contract and release part of the water previously enclosed. This phenomenon did not occur in samples with higher *Psyllium* gel ratios. A recent study by Figueroa and co-workers [21] reported the positive influence of *Psyllium* on the absence of syneresis of fruit jellies enriched with dietary fiber.

In Table 1, a chemical characterization of *Psyllium* husk and the developed gels is presented.

Table 1. Moisture, ash, and sulfate content of *Laminaria ochroleuca*, *Psyllium* husk, and the developed gels.

Samples	Moisture	Ash	Sulphates
	(%)	(%, d.b.)	
***Laminaria ochroleuca* [17]**	9.20 ± 0.07	35.01 ± 0.31	2.21 ± 0.10
***Psyllium* husk (160–315 μm)**	9.03 ± 0.31 h	2.98 ± 0.05 g	0.09 ± 0.00 e
Psy (control)	95.83 ± 0.08 a	2.91 ± 0.18 g	0.20 ± 0.00 e
PsyL	93.58 ± 0.06 b	18.01 ± 1.83 f	0.98 ± 0.00 d
Lo.Psy_25.75	91.73 ± 0.03 c	24.75 ± 0.51 d	1.75 ± 0.01 c
Lo.Psy_50.50	87.50 ± 0.06 e	33.08 ± 0.24 b	2.20 ± 0.07 a,b
Lo.Psy_75.25	84.48 ± 0.22 g	36.02 ± 0.23 a	1.98 ± 0.13 b,c
LoUS.Psy_25.75	91.66 ± 0.12 c	21.91 ± 0.71 e	1.78 ± 0.02 c
LoUS.Psy_50.50	87.78 ± 0.13 d	30.34 ± 0.41 c	2.14 ± 0.11 a,b
LoUS.Psy_75.25	85.18 ± 0.12 f	34.71 ± 0.37 a,b	2.25 ± 0.01 a

Psy (control), *Psyllium* gels prepared in water; PsyL, *Psyllium* gels prepared in autohydrolysis liquor; *Laminaria-Psyllium* gels: Lo.Psy_25.75, Lo.Psy_50.50, and LoPsy_75.25; *Laminaria* with ultrasonic treatment-*Psyllium* gels: LoUS.Psy_25.75, LoUS.Psy_50.50, and LoUSPsy_75.25. Data are presented as the mean ± sd. Different letters in a column show significantly different data values at the $p < 0.05$ level.

The moisture content of the *Laminaria-Psyllium* gels depends greatly on the proportion of both components, and decreased with the increase in *Laminaria* content. Ash content revealed the reverse trend, with the *Laminaria* proportion being crucial for the final gel mineral content. This is due to the high ash content (35%, d.b.) of this alga, as we previously determined [17]. It is also noteworthy that the ultrasonic treatment applied to the purée-like mixtures had a significant ($p < 0.05$) negative influence on the total ash content of the gel, especially in the 25.75 and 50.50 samples.

Sulfate presence in the *Laminaria-Psyllium* gels is a clear indication of the presence of fucoidan, a sulfated polysaccharide with reported activity against stomach-gastric adenocarcinoma cells and lung carcinoma cells [22].

The most important chemical feature of *Psyllium* husk is its high soluble fiber content (80%, d.b., as previously determined by the authors in the same sample [10]. However, aside from its fiber content, *Psyllium* husk has a mineral composition of 10.3 g K/kg, 0.88 g Na/kg, 1.36 g Ca/kg, and 94.7 mg Fe/kg, (present study), the last two minerals having a higher content than most cooked pulses [23].

Another important feature is *Psyllium*'s ability to retain sodium at physiological important conditions (pH 1.2—stomach; pH 6.8—intestine), being potentially active in reducing the bioavailable fraction of ingested sodium in the body [24].

Considering both the *L. ochroleuca* [17] and *Psyllium* husk's mineral content, one can conclude that the partnership between this alga and *Psyllium* husk could be advantageous for the development of new food products.

Water activity was very high and ranged between 0.999–1.000 for all samples (data not shown) and these are typical values for gels, although some reduction was expected in gels with a high proportion of *Psyllium*.

3.2. Color Evaluation of Samples

From the color evaluation results presented in Table 2, the *Psyllium* gels (control and PsyL) only differed in terms of their chromatic parameters, which could be of importance depending on the desired application.

Table 2. Color parameters (L*, a*, b*) and ΔE* of *Psyllium* husk, *Laminaria ochroleuca* and its AH liquor, and the gels developed.

Samples	L *	a *	b *	ΔE *
***Psyllium* husk (160–315 μm)**	59.39 ± 0.91 a	6.06 ± 0.18 a	24.22 ± 0.37 a	-
***Laminaria ochroleuca* [17]**	56.36 ± 0.57	−2.48 ± 0.04	14.49 ± 0.18	-
AH liquor [17]	31.42 ± 1.74	1.45 ± 0.26	2.88 ± 1.60	-
Psy (control)	33.12 ± 1.78 b,c	1.32 ± 0.14 c	9.85 ± 1.36 c	-
PsyL	35.70 ± 2.86 b	3.78 ± 1.50 b	17.67 ± 5.54 b	8.6
Lo.Psy_25.75	30.08 ± 0.37 c,d	−0.16 ± 0.22 d	12.82 ± 0.57 c	4.5
Lo.Psy_50.50	27.50 ± 0.77 d,e	−0.07 ± 0.16 d	12.37 ± 1.07 c	6.3
Lo.Psy_75.25	25.14 ± 1.70 e	−0.45 ± 0.09 d	10.90 ± 1.24 c	8.2
LoUS.Psy_25.75	29.98 ± 0.82 d	−0.06 ± 0.23 d	14.10 ± 0.46 b,c	5.5
LoUS.Psy_50.50	26.96 ± 1.17 d,e	−0.40 ± 0.09 d	12.38 ± 1.15 c	6.9
LoUS.Psy_75.25	25.87 ± 0.55 e	−0.64 ± 0.06 d	11.60 ± 1.14 c	7.7

Psy (control), *Psyllium* gels prepared in water; PsyL, *Psyllium* gels prepared in autohydrolysis liquor; *Laminaria-Psyllium* gels: Lo.Psy_25.75, Lo.Psy_50.50, and LoPsy_75.25; *Laminaria* with ultrasonic treatment-*Psyllium* gels: LoUS.Psy_25.75, LoUS.Psy_50.50, and LoUSPsy_75.25. Data are presented as the mean ± sd. Different letters in the same column correspond to significant differences ($p < 0.05$).

As expected, the lightness (L*) of the gel samples decreased with the incorporation of *Laminaria*, although significant differences were only registered between the *Laminaria-Psyllium* 25.75 and *Laminaria-Psyllium* 75.25 samples. It is noteworthy that the color parameters of the gel samples with the highest *Laminaria* content were like those obtained in the purée-like mixtures [14]. Moreover, the color parameters L* (Lo.Psy: r = −0.928, $p = 1.3 \times 10^{-9}$; LoUS.Psy: r = −0.920, $p = 9.3 \times 10^{-9}$) and a* (Lo.Psy: r = −0.849; $p = 1.2 \times 10^{-6}$; LoUS.Psy: r = −0.913, $p = 2.0 \times 10^{-8}$) were strongly negatively correlated with *Laminaria* content in the gel formulations.

The ΔE* between the *Laminaria-Psyllium* gels and the control ranged from 4.5 to 8.2, increasing with the increase in the *Laminaria* content in the system. These values indicate that the consumer would distinguish between the two samples compared [25].

It should be pointed out that the gels proposed here are not finished products. In this sense, the color of the final food gel product could be optimized by considering these findings and according to the desired final color.

3.3. Effect of Laminaria-Psyllium Ratio on the Dynamic Viscoelasticity

Figure 2 shows the elastic behavior of *Laminaria-Psyllium* gel systems. As the alga fraction increased, a structuring effect was observed in the gels. This behavior was markedly noticed in the highest alga concentration (Lo.Psy_75.25 and LoUS.Psy_75.25). This interaction is probably due to physical entanglements between the polymers present in both the alga and *Psyllium*, causing the reinforcement of the gel. However, in the absence of the *Psyllium* gel, the alga purées showed a huge decrease in G′ to values similar to the control (Psy), but more frequency dependent (b′ = 0.209) [17].

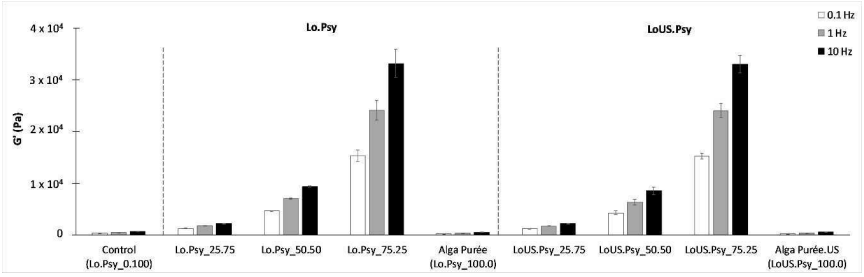
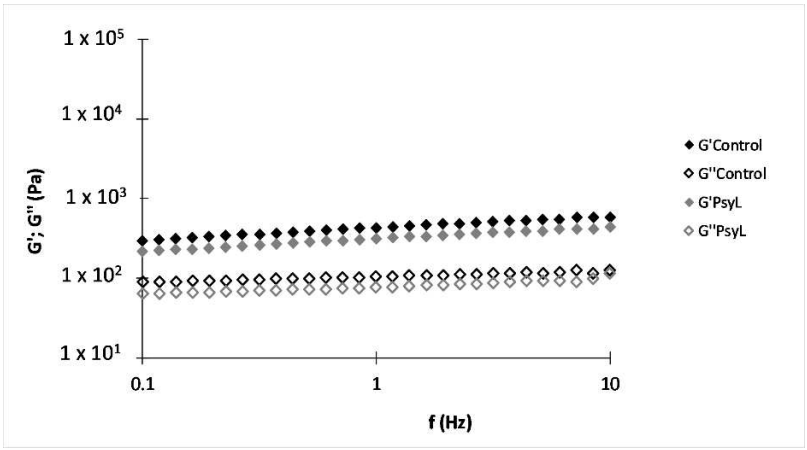


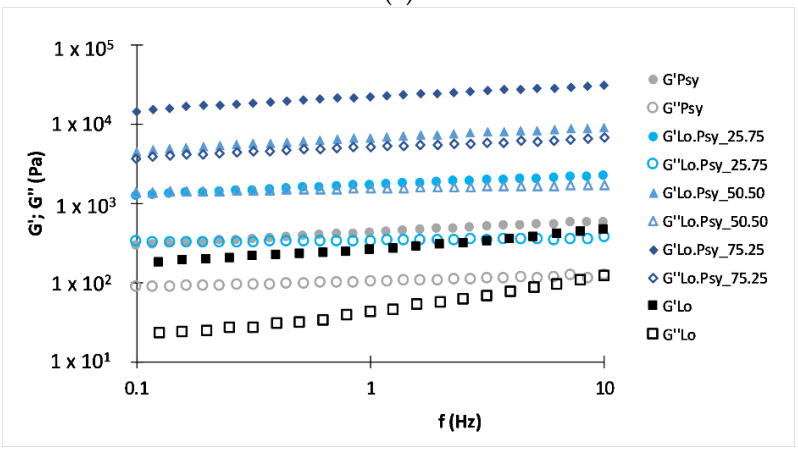
Figure 2. Elastic modulus at 0.1 Hz, 1 Hz, and 10 Hz of *Laminaria-Psyllium* gels with (LoUS.Psy) and without ultrasonic treatment (Lo.Psy), control, and *Laminaria* purées (Alga Purée; Alga Purée.US) [17].

This synergistic interaction was also found in other polymeric systems, namely between the locust bean gum (LBG) and xanthan gum, where the latter did not form gel, rather a shear-thinning solution, but combined with LBG to form a strong gel structure [26].

The mechanical spectra of the developed gels are depicted in Figure 3.



(**a**)



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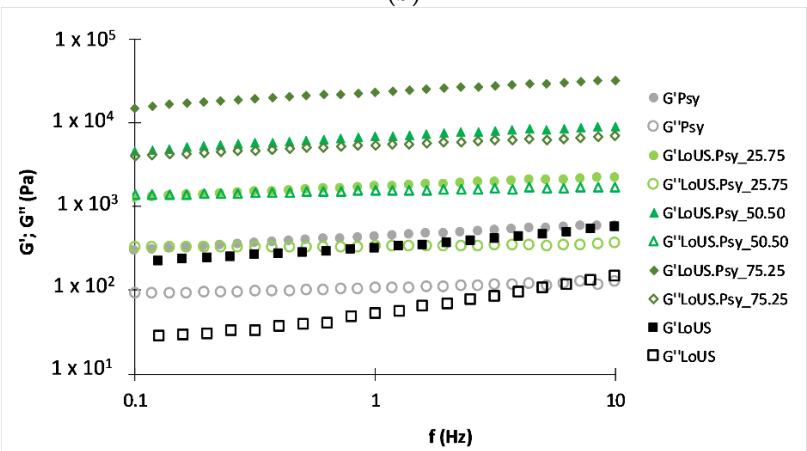


(**c**)

Figure 3. Mechanical spectra of the *Psyllium* gels prepared in water (control) and autohydrolysis liquor (PsyL).(**a**) *Laminaria-Psyllium* gels (Lo.Psy_25.75, Lo.Psy_50.50, LoPsy_75.25); (**b**) *Laminaria* with ultrasonic treatment-*Psyllium* gels (LoUS.Psy_25.75, LoUS.Psy_50.50, LoUSPsy_75.25; (**c**) G', closed symbol; G", open symbol.

To quantify the impact of the different combinations of *Laminaria* and *Psyllium* on the viscoelastic moduli, the variation of G′ and G″ with gel composition was obtained from the respective mechanical spectra (Table 3).

Table 3. Power law parameters (α′, α″, b′, and b″) of the gel samples with *Laminaria ochroleuca* and *Psyllium* husk, control, and PsyL.

Samples	G′		G″	
	α′	b′	α″	b″
Psy (Control)	436.8 ± 15.4	0.153 ± 0.002	104.8 ± 7.0	0.071 ± 0.011
PsyL	323.0 ± 18.2	0.149 ± 0.005	79.6 ± 2.4	0.090 ± 0.005
Lo.Psy_25.75	1689.3 ± 20.8	0.124 ± 0.000	344.2 ± 2.7	0.022 ± 0.000
Lo.Psy_50.50	6819.9 ± 103.8	0.148 ± 0.002	1584.5 ± 28.3	0.049 ± 0.002
Lo.Psy_75.25	0.5 ± 1816.6	0.158 ± 0.002	5485.0 ± 434.5	0.119 ± 0.001
LoUS.Psy_25.75	1681.6 ± 3.5	0.121 ± 0.000	331.9 ± 2.8	0.019 ± 0.000
LoUS.Psy_50.50	6239.3 ± 543.3	0.146 ± 0.002	1442.5 ± 122.0	0.049 ± 0.001
LoUS.Psy_75.25	23,533.5 ± 1267.8	0.159 ± 0.001	5530.1 ± 331.8	0.116 ± 0.002

The goodness of fitting (R^2) ranged from 0.995–0.997 for G′ and from 0.936–0.997 for G″.

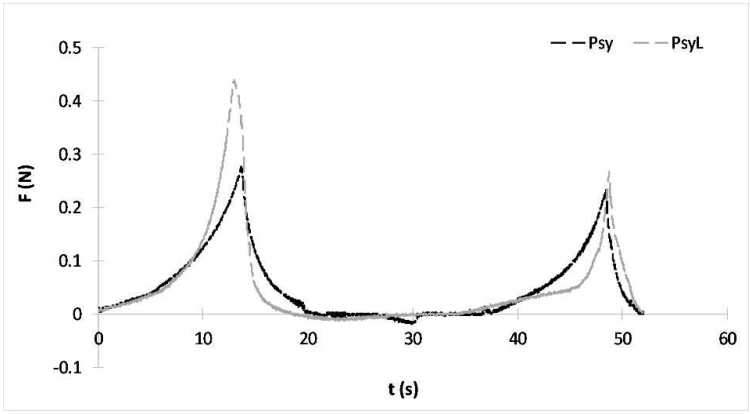
The mechanical spectra Psy (control) and PsyL exhibited similar viscoelastic performance typical of well-structured weak gels, with slight frequency dependence (Figure 3a). This result is consistent with the rheological study by Haque et al. [27], in which it is also reported that *Psyllium* husk forms gel even at low temperature, this being an important feature to consider in food design. Moreover, the developed gels were more stable at higher frequencies than the ones produced with 10–15% chia flour at 90 °C (Ramos et al., 2016), reinforcing *Psyllium*'s potential as a valuable and sustainable biopolymer.

As mentioned earlier, PsyL appeared to be more fluid than the control (Psy), which was confirmed by the mechanical spectra, with both viscoelastic moduli of the control being higher than those of PsyL, and by the decrease of α′ (Table 3). Autohydrolysis promotes the solubilization of minerals [17] and the depolymerization of polysaccharides, namely alginate, fucoidan, and laminarin present in brown algae [28], rendering a liquid extract with an acidic pH (≈5, [19]). Since *Laminaria* liquid extract is a multicomponent matrix, its effect on the *Psyllium* gel properties are more complex, depending not only on the solution pH, but also on the type of ions in the solution, and even on the presence of peptides and other molecules resulting from the depolymerization of the polysaccharides. These polymer fractions can interfere with the gel matrix and exert an antagonism, leading to the reduction of gel links and de-structuring the material.

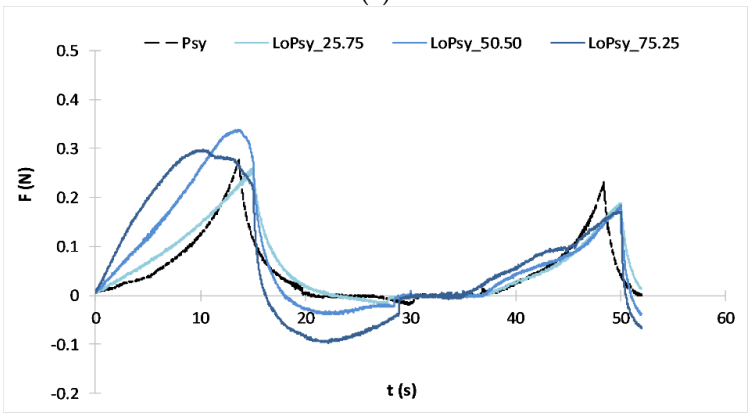
The ultrasonic pre-treatment applied to *L. ochroleuca* did not affect the rheological behavior of the *Laminaria-Psyllium* gels. The weak-gel like behavior was maintained in all *Laminaria-Psyllium* gel samples, and was more noticeable as the alga fraction increased, as can be observed by the increase in the b′ parameter of the resulting power law (Table 3). As the incorporation of the alga solid fraction increased, the dependence of the material on frequency increased; however the value of the viscoelastic moduli increased probably due to the reduction of the number of links, but also stronger ones, which may be due to interactions between the alginate from the alga and fiber from the *Psyllium* husk reinforced by calcium ions [29].

3.4. Texture Properties of the Laminaria-Psyllium Gel Systems

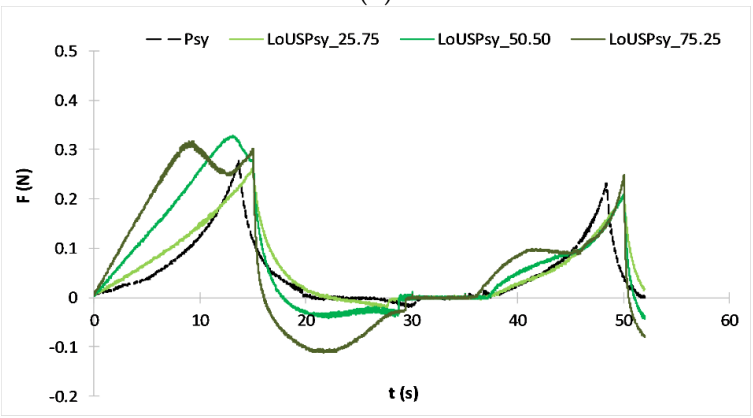
Texture is a major quality parameter, and is crucial for consumer acceptance. Figure 4 presents the texture profiles of the samples and the commercial references.



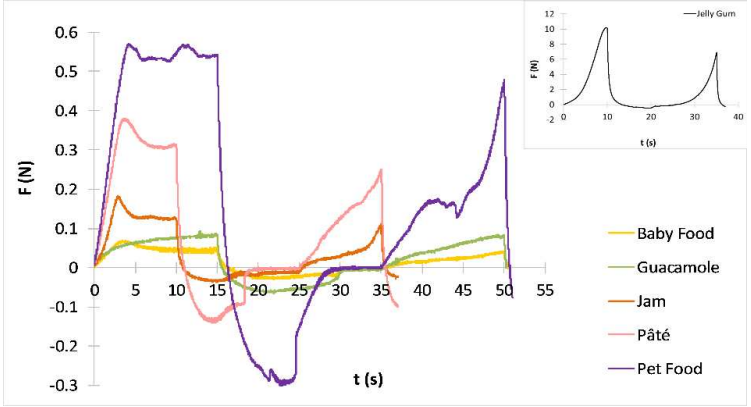
(**a**)



(**b**)



(**c**)



(**d**)

Figure 4. Texture profiles of Psy (control) and PsyL (**a**), *Laminaria-Psyllium* gels (**b**), *Laminaria-Psyllium* gels subjected to ultrasonic treatment (**c**), and commercial products (**d**). The differences in the texturograms reflect the different sizes and shapes of the commercial products.

Each food system (e.g., jelly, mayonnaise, and baby food) presents texture properties that are specific, easily recognizable, and desired by the consumers. Despite the information given by the texture profile, the use of this methodology is very limited in the scientific literature. To our knowledge, no published studies refer to the texture features of commercial gelled products, therefore we show here some texture profiles of different commercial food products to illustrate the data for these systems to be used as a target for further product development. Based on these texture profiles (Figure 4d), three groups of samples can be discriminated: one composed of *Pâté*, *Pet Food* and *Jam*; another with *Baby Food* and *Guacamole*; and a third with *Jelly Gum*, whose graph is shown separately. In the first group of samples, the rupture point occurred at a low break distance, and the force reached a plateau until the probe retracted from the sample. This mechanical behavior was similar to what we found in LoUS.Psy_75.25 (Figure 4c) and has also been reported by Genovese and co-workers [30] for pectin gels. The control and PsyL texture profiles (Figure 4a) were similar to the one obtained by Figueroa et al. [21] for fruit jellies with *Psyllium*, although with much lower magnitude.

From the texture profiles depicted, the firmness, adhesiveness, and cohesiveness values were calculated (Table 4).

Table 4. Texture parameters (firmness, adhesiveness and cohesiveness) of the gels developed and commercial references.

Samples		Firmness (N)	Adhesiveness (N.s)	Cohesiveness
Developed gel samples	**Psy (control)**	0.296 ± 0.036 c,d,e	−0.034 ± 0.006 a	0.581 ± 0.051 b
	PsyL	0.419 ± 0.031 b,c	−0.032 ± 0.003 a	0.453 ± 0.024 c,d,e
	Lo.Psy_25.75	0.259 ± 0.028 c,d,e	−0.040 ± 0.012 a	0.517 ± 0.037 b,c
	Lo.Psy_50.50	0.316 ± 0.020 c,d,e	−0.274 ± 0.038 a	0.362 ± 0.044 e,f,g
	Lo.Psy_75.25	0.279 ± 0.039 c,d,e	−0.977 ± 0.075 c	0.403 ± 0.026 d,e,f
	LoUS.Psy_25.75	0.261 ± 0.007 c,d,e	−0.069 ± 0.017 a	0.538 ± 0.009 b,c
	LoUS.Psy_50.50	0.333 ± 0.026 b,c,d	−0.268 ± 0.042 a	0.354 ± 0.028 f,g
	LoUS.Psy_75.25	0.319 ± 0.024 c,d,e	−0.978 ± 0.047 c	0.391 ± 0.030 d,e,f,g
Commercial products	**Baby Food**	0.065 ± 0.008 d,e	−0.325 ± 0.034 a,b	0.475 ± 0.023 c,d
	Jelly Gum	10.314 ± 0.386 a	−3.596 ± 0.562 e	0.330 ± 0.028 f,g
	Guacamole	0.091 ± 0.011 e	−0.638 ± 0.106 b,c	0.748 ± 0.075 a
	Jam	0.182 ± 0.003 c,d,e	−0.298 ± 0.015 a	0.310 ± 0.015 g
	Pâté	0.364 ± 0.032 b,c	−0.854 ± 0.081 c	0.464 ± 0.038 c,d
	Pet Food	0.587 ± 0.066 b	−2.173 ± 0.148 d	0.371 ± 0.024 e,f,g

Psy (control), *Psyllium* gels prepared in water; PsyL, *Psyllium* gels prepared in autohydrolysis liquor; *Laminaria-Psyllium* gels: Lo.Psy_25.75, Lo.Psy_50.50, and LoPsy_75.25; *Laminaria* with ultrasonic treatment-*Psyllium* gels: LoUS.Psy_25.75, LoUS.Psy_50.50, and LoUSPsy_75.25. Data are presented as the mean ± sd. Different letters in the same column correspond to significant differences ($p < 0.05$).

In general, it can be said that the firmness of the *Laminaria-Psyllium* system is independent of the level of *Laminaria* incorporation and the ultrasonic (US) pre-treatment. On the other hand, there are strong negative correlations between the adhesiveness ($r = -0.888$, $p < 0.05$) and cohesiveness ($r = -0.844$, $p < 0.05$) parameters and *Laminaria* concentration, and again, the US pre-treatment did not make any change. The fact that adhesiveness can be adjusted by keeping the firmness values constant could be useful in product development.

In a previous study by the authors [17], it was found that the texture parameters, especially adhesiveness, of the alga purée-like mixtures varied according to the ultrasonic treatment. However, that difference was not maintained after the addition of *Psyllium*. Since PsyL did not show adhesiveness values that could explain this result, one can conclude that there is a synergistic effect of *Laminaria* and *Psyllium* in this texture parameter, stronger than mere addition. Based on these results and looking at the texture profiles of the samples (Figure 4a–c), one can conclude that the texture properties of *Laminaria-Psyllium* gels are governed by the alga, what is in agreement with the dynamic rheology results. Texture profiles (Figure 4) confirm the relevance of alga in the system.

The texture parameters of the commercial products varied greatly. Comparing the texture parameters of the gelled systems developed with those of the references (Table 4), the developed *Laminaria-Psyllium* gels presented similar values to the commercial products. Although the systems developed are not finished products, but binary systems consisting of alga purée and *Psyllium* gel, this is a good starting point for the development of enriched gelled food products. These *Laminaria* and *Psyllium* whole materials can be used as alternatives to the biopolymers that are usually added to build up structure in foods. This is in line with the food trends regarding the use of natural products over processed ones.

It should be pointed out that in this approach, we developed the *Laminaria-Psyllium* gels using water, but the gels could also be produced in the same way using the AH liquor extract, thus taking advantage of the soluble compounds present there, namely phenolic compounds with antioxidant capacity [17]. Due to the differences in texture and rheological features between the control and PsyL, it is plausible to assume that *Laminaria-Psyllium* gels prepared in AH liquor would present distinct mechanical properties.

4. Conclusions

Food gels were developed with *Laminaria* and *Psyllium* husk, adding value to both these natural resources. *Psyllium* husk interacted positively with this alga, reinforcing the viscoelastic behavior of the obtained gels. These novel functional gels with minimal processing can be a starting point to the development of several food and feed applications.

Food application studies are in progress to evaluate the demonstrated potential of this *Laminaria-Psyllium* partnership.

Author Contributions: P.F., M.D.T., and A.R. conceived and planned the experiments. P.F. and M.D.T. participated in the sample preparation and analysis, data analysis and interpretation of the results, and writing of the manuscript with input from all authors. I.S., A.R., and H.D. supervised the research work, contributed to the discussion of the data, and revised the manuscript. All authors contributed with suggestions and comments for the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kim, S.-K.; Bhatnagar, I. Physical, Chemical, and Biological Properties of Wonder Kelp—*Laminaria*. In *Advances in Food and Nutrition Research*; Kim, S.-K., Ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2011; pp. 85–96. [CrossRef]
2. Adebisi, A.O.; Laity, P.R.; Conway, B.R. Formulation and evaluation of floating mucoadhesive alginate beads for targeting *Helicobacter pylori*. *J. Pharm. Pharmacol.* **2015**, *67*, 511–524. [CrossRef] [PubMed]
3. Oliveira, S.; Fradinho, P.; Mata, P.; Moreira-Leite, B.; Raymundo, A. Exploring innovation in a traditional sweet pastry: Pastel de Nata. *Int. J. Gastron. Food Sci.* **2019**, *17*, 100160. [CrossRef]
4. Sánchez-Machado, D.I.; López-Cervantes, J.; López-Hernández, J.; Paseiro-Losada, P. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.* **2004**, *85*, 439–444. [CrossRef]
5. Fernandes, F.; Barbosa, M.; Oliveira, A.P.; Azevedo, I.C.; Sousa-Pinto, I.; Valentão, P.; Andrade, P.B. The pigments of kelps (*Ochrophyta*) as part of the flexible response to highly variable marine environments. *J. Appl. Phycol.* **2016**, *28*, 3689–3696. [CrossRef]
6. Parada, J.; Pérez-Correa, J.R.; Pérez-Jiménez, J. Design of low glycemic response foods using polyphenols from seaweed. *J. Funct. Foods.* **2019**, *56*, 33–39. [CrossRef]
7. Andrade, P.B.; Barbosa, M.; Matos, R.P.; Lopes, G.; Vinholes, J.; Mouga, T.; Valentão, P. Valuable compounds in macroalgae extracts. *Food Chem.* **2013**, *138*, 1819–1828. [CrossRef]

8. Roohinejad, S.; Koubaa, M.; Barba, F.J.; Saljoughian, S.; Amid, M.; Greiner, M. Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Res. Int.* **2017**, *99*, 1066–1083. [CrossRef]
9. FDA. CFR–code of federal regulations title 21. U.S. Food and Drug Administration. 2012. Available online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.81 (accessed on 26 July 2019).
10. Raymundo, A.; Fradinho, P.; Nunes, M.C. Effect of *Psyllium* fibre content on the textural and rheological characteristics of biscuit and biscuit dough. *Bioact. Carbohydr. Dietary Fibre.* **2014**, *3*, 96–105. [CrossRef]
11. Fischer, M.H.; Yu, N.; Gray, G.R.; Ralph, J.; Anderson, L.; Marlett, J.A. The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk). *Carbohyd. Res.* **2004**, *339*, 2009–2017. [CrossRef]
12. Askari, F.; Sadeghi, E.; Mohammadi, R.; Rouhi, M.; Taghizadeh, M.; Shirgardoun, M.H.; Kariminejad, M. The physicochemical and structural properties of psyllium gum/modified starch composite edible film. *J. Food Process Preserv.* **2018**, *42*, 13715. [CrossRef]
13. Singh, B. Psyllium as therapeutic and drug delivery agent. *Int. J. Pharm.* **2007**, *334*, 1–14. [CrossRef] [PubMed]
14. Ramos, S.; Fradinho, P.; Mata, P.; Raymundo, A. Assessing gelling properties of chia (*Salvia hispanica L.*) flour through rheological characterization. *J. Sci. Food Agric.* **2016**, *97*, 1753–1760. [CrossRef] [PubMed]
15. Soukoulis, C.; Cambier, S.; Serchi, T.; Tsevdou, M.; Gaiani, C.; Ferrer, P.; Taoukis, P.S.; Hoffmann, L. Rheological and structural characterisation of whey protein acid gels co-structured with chia (*Salvia hispanica L.*) or flax seed (*Linum usitatissimum L.*) mucilage. *Food Hydrocolloid.* **2019**, *89*, 542–553. [CrossRef]
16. Soukoulis, C.; Gaiani, C.; Hoffmann, L. Plant seed mucilage as emerging biopolymer in food industry applications. *Curr. Opin. Food Sci.* **2018**, *22*, 28–42. [CrossRef]
17. Fradinho, P.; Flórez-Fernández, N.; Sousa, I.; Raymundo, A.; Domínguez, H.; Torres Pérez, M.D. Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties. *J. Appl. Phycol.* **2019**. accepted for publication.
18. Fradinho, P.; Sousa, I.; Raymundo, A. The role of *Psyllium* gels on the structuring of gluten-free fresh pasta –a rheological approach. In *Book of Abstracts of AERC 2019. Proceedings of the Oral Communication FP11, Portoröz, Slovenia, 8–11 April 2019*; The European Society of Rheology: Zurich, Switzerland, 2019; p. 29.
19. Gómez-Ordóñez, E.; Jiménez-Escrig, A.; Rupérez, P. Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Res. Int.* **2010**, *43*, 2289–2294. [CrossRef]
20. Paraskevopoulou, A.; Kiosseoglou, V.; Alevisopoulos, S.; Kasapis, S. Small deformation properties of model salad dressings prepared with reduced cholesterol egg yolk. *J. Texture Stud.* **1997**, *28*, 221–237. [CrossRef]
21. Figueroa, L.E.; Genovese, D.B. Fruit jellies enriched with dietary fibre: Development and characterization of a novel functional food product. *LWT Food Sci. Technol.* **2019**, *111*, 423–428. [CrossRef]
22. Flórez-Fernández, N.; Torres, M.D.; González-Muñoz, M.J.; Domínguez, H. Recovery of bioactive and gelling extracts from edible brown seaweed *Laminaria ochroleuca* by non-isothermal autohydrolysis. *Food Chem.* **2019**, *277*, 353–361. [CrossRef]
23. Margier, M.; Georgé, S.; Hafnaoui, N.; Remond, D.; Nowicki, M.; Du Chaffaut, L.; Amiot, M.-J.; Reboul, E. Nutritional Composition and Bioactive Content of Legumes: Characterization of Pulses Frequently Consumed in France and Effect of the Cooking Method. *Nutrients* **2018**, *10*, 1668. [CrossRef]
24. Jimoh, M.A.; MacNaughtan, W.; Williams, H.E.L.; Greetham, D.; Linforth, R.L.; Fisk, I.D. Sodium ion interaction with psyllium husk (*Plantago sp.*). *Food Funct.* **2016**, *7*, 4041. [CrossRef] [PubMed]
25. Castellar, M.R.; Obón, J.M.; Fernández-López, J.A. The isolation and properties of a concentrated red-purple betacyanin food colorant from *Opuntia stricta* fruits. *J. Sci. Food Agric.* **2006**, *86*, 122–128. [CrossRef]
26. Copetti, G.; Grassi, M.; Lapasin, R.; Pricl, S. Synergistic gelation of xanthan gum with locust bean gum: A rheological investigation. *Glycoconj. J.* **1997**, *14*, 951–961. [CrossRef] [PubMed]
27. Haque, A.; Richardson, R.K.; Morris, E.R.; Dea, I.C.M. Xanthan-like 'weak gel' rheology from dispersions of ispaghula seed husk. *Carbohyd. Polym.* **1993**, *22*, 223–232. [CrossRef]
28. Rodríguez-Jasso, R.M.; Mussatto, S.I.; Pastrana, L.; Aguilar, C.N.; Teixeira, J.A. Extraction of sulfated polysaccharides by autohydrolysis of brown seaweed *Fucus vesiculosus*. *J. Appl. Phycol.* **2013**, *25*, 31–39. [CrossRef]

29. Guo, Q.; Cui, S.W.; Wang, Q.; Goff, H.D.; Smith, A. Microstructure and rheological properties of psyllium polysaccharide gel. *Food Hydrocolloid.* **2009**, *23*, 1542–1547. [CrossRef]
30. Genovese, D.B.; Ye, A.; Singh, H. High Methoxyl Pectin/Apple Particles Composite Gels: Effect of Particle Size and Particle concentration on Mechanical Properties and Gel Structure. *J. Text Stud.* **2010**, *41*, 171–189. [CrossRef]

Chapter 5. Incorporation of bioactive sources into gluten-free pasta

The work presented in this chapter was included in the following publications:

- ***Research Paper 5***
  Fradinho, P., Sousa, I., Raymundo, A., Domínguez, H., Torres Pérez, M.D. (2019). Edible brown seaweed in gluten-free pasta: technological and nutritional evaluation. *Foods*, 8(12), 622. https://doi.org/10.3390/foods8120622 (Impact Factor: 3.011, Q2)

- ***Research Paper 6***
  Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A. (2020). Effect of *Arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research*, 45, 101743. https://doi.org/10.1016/j.algal.2019.101743 (Impact Factor: 3.723, Q1)

- ***Research Paper 7***
  Fradinho, P., Oliveira, A., Sousa, I., Raymundo, A., Domínguez, H., Torres, M.D. (2020). Improving the nutritional performance of gluten-free pasta with potato peel autohydrolysis extract. Innovative Food Science and Emerging Technologies, 63, 102374. https://doi.org/10.1016/j.ifset.2020.102374 (Impact Factor: 4.085, Q1)

In Figure E the schematic summary of the chapter 5 is presented.

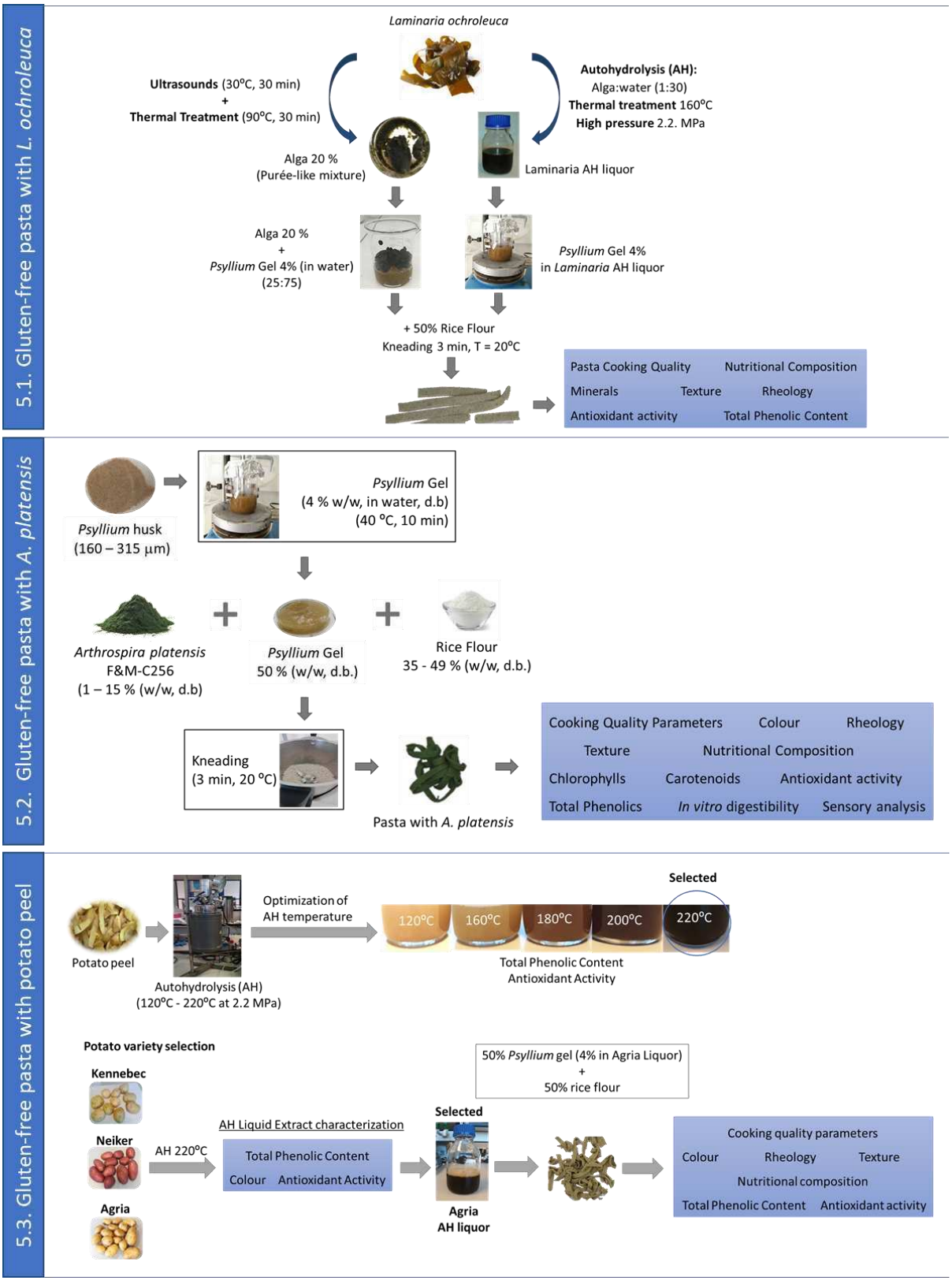

Figure E. Schematic procedure of chapter 5.

foods

MDPI

Article

Edible Brown Seaweed in Gluten-Free Pasta: Technological and Nutritional Evaluation

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Abstract: Seaweed is a novel source of important nutritional compounds with interesting biological activities that could be processed into added-value products, namely gluten-free foods. In this study, two previously developed products obtained from *Laminaria ochroleuca* processing (liquid extract and a purée-like mixture) were incorporated in gluten-free (GF) pasta in order to develop functional products especially designed for the celiac population. The raw and cooked pastas were characterized in terms of their cooking quality parameters, nutritional composition, texture and rheological properties, and antioxidant activity. It was found that the developed GF pastas had similar mechanical and texture characteristics to the control. Both supplemented GF pastas presented a significantly ($p < 0.05$) higher fibre and mineral content than the control pasta.

Keywords: kombu; *Psyllium*; *Laminaria ochroleuca*; by-products; celiac, cooking quality; antioxidants; fibre; minerals

1. Introduction

Innovative food products with health-promoting components have gained growing interest within the research community, food industry, and consumers [1–4]. The gluten-free (GF) foods available in the market are nutritionally unbalanced, promoting high intake of energy, sugars, lipids, and a low intake of fibre, vitamins (B group and D), and minerals (Ca, Fe, Mg, and Zn) [5,6]. Besides nutritional deficiencies, celiac consumers consider GF foods less appealing in terms of texture and flavour than similar products with gluten [7].

Seaweed is a natural source of interesting compounds for human nutrition, such as fatty acids, proteins, minerals [8], vitamins (A, C, D, B group, E, K, PP) [9], and pigments such as carotenoids and polyphenols, with proven antioxidant, hypoglycaemic, antitumoral, and antimicrobial activities [10–12].

Although their consumption is not massified, their popularity has increased, especially amongst vegetarians and consumers looking for new food sources and tastes [13]. A challenge for the coming years is to introduce seaweed or their high-valuable extracts as additives in food matrices. Due to its high content in biologically active compounds, seaweed, especially Phaeophyta (brown algae), has great potential to be used in food applications [14,15]. However, despite the interest of the research community and the flourishing availability of Asian food, the current European retail market of seaweed food products is limited to canned food, salad dressings, and pâtés, among others [16,17].

Laminaria ochroleuca is a perennial warm-water temperature brown seaweed, distributed from the south of England to Morocco [18]. It has interesting biochemical composition [19] and could enhance

the nutritional profile of food products targeted for populations with specific nutritional requirements such as the ones of the celiac population and consumers with some type of wheat allergy.

Several studies of seaweed incorporation and/or their extracts into several food matrices (meat, pasta, cheese) have been performed [20–22] to enhance nutritional and texture features, antioxidant capacity, or shelf life stability of the product. Regardless of the potential already described, to our knowledge, there are no available studies addressing the supplementation of GF foods with seaweed.

Processing conditions modify the nutritional and mechanical profile of foods, either by contributing to the acquisition of desirable features (e.g., crunchiness), or to the loss of antinutrient phytochemicals, as well as some losses of vitamins and minerals [23]. Moreover, processing methods improve protein and carbohydrate digestibility of foods [24–26]. Therefore, the assessment of the actual nutritional and phytochemical composition of foods is a mandatory issue for the design of functional foods for populations with specific requirements.

This study aims to add value to *L. ochroleuca* brown algae, through its incorporation as a high-valuable additive in functional GF pasta. The raw and cooked GF pastas were extensively characterized in terms of physical properties and biochemical composition, in order to assess the actual nutrient intake.

2. Materials and Methods

2.1. Raw Materials

Dehydrated *Laminaria ochroleuca* (Algas Atlánticas Algamar, S.L., lot B39137547, Pontevedra, Spain) was milled and sieved to obtain powders with <0.25 mm and 0.25–2 mm diameter particle sizes. Rice flour (Ceifeira, Dacsa Atlantic, lot 3411/18, Lisboa, Portugal) and *Psyllium* husk of Indian origin (Solgar, lot 107028-01, Leonia, NJ, USA) were purchased in a local market. *Psyllium* was milled and sieved to 160–315 µm (Pulverisette 14 Premium, Fritsch, Idar-Oberstein, Germany).

2.2. Fresh Pasta Preparation and Sampling

Focusing the full valorisation of *L. ochroleuca*, two samples were prepared for pasta incorporation: (1) autohydrolysis (AH) liquid extract obtained according to the procedure described in Fradinho et al. [19]; (2) purée-like mixture (20% (*w/w*) alga, ultrasound 30 min, 90 °C/30 min prepared following the procedure already described by Fradinho et al. [19] (Figure 1).

Then, both the AH liquid extract and the purée samples were processed with *Psyllium* husk or *Psyllium* gel (4% *w/w*, dry basis (d.b.)) [27] to obtain *Laminaria–Psyllium* gels. The selection of *Laminaria–Psyllium* 25:75 ratio was on the basis of absence of syneresis and good mechanical properties assessed in a previous study by the authors [28].

Three batches of 200 g of each pasta formulation were prepared by mixing each *Laminaria–Psyllium* gel with rice flour (thermoprocessor: Bimby TM31, Vorwerk, Wuppertal, Germany), at a 50:50 ratio, at room temperature for 3 min.

The dough was sheeted and laminated as tagliatelle (width = 6.10 mm, thickness = 2.12 mm, length = 70 cm) using a benchtop pasta machine (Atlas 150 Wellness, Marcato, Campodarsego, Italy). Then, the pasta dough was covered with aluminium foil and placed in an air oven at 25 °C for 15 min to ensure structure recovery. A control sample was also prepared using only rice flour and *Psyllium* gel 4 % (*w/w*).

Cooking quality of pasta (optimal cooking time (OCT), water absorption, swelling power, and cooking loss) was assessed on raw samples following the procedure described in AACC 66–50 [29] and the methods described in Fradinho et al. [30]. Each determination was performed at least three times.

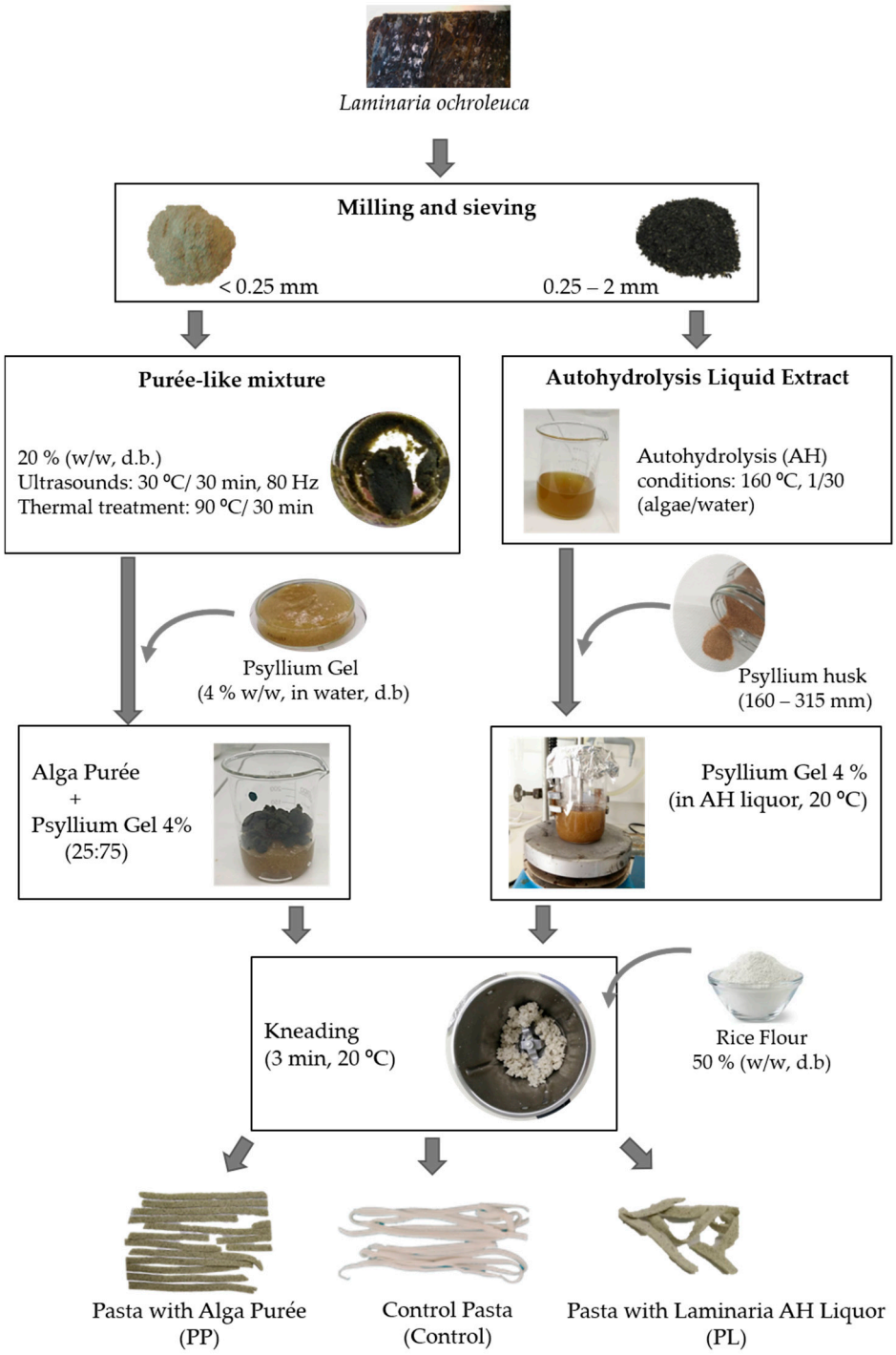


Figure 1. Schematic procedure of pasta preparation. dry basis: (d.b.)

The pasta samples (uncooked and cooked) destined for physicochemical and bioactive analysis were lyophilized at −55 °C for 72 h (Scanvac Coolsafe 55-4, Labogene, Allerød, Denmark), crushed into powder (<0.5 mm), and stored at room temperature until biochemical analysis. Physical analyses (colour, texture, and rheology) were performed on fresh samples immediately after pasta resting.

2.3. Colour Evaluation of Pasta

The colour measurements of raw and cooked pasta samples were performed instrumentally using a CR-400 colorimeter (Minolta, Tokyo, Japan) with standard illuminant D65 and a visual angle of 2°. For calibration purposes, a white standard was used (L* = 97.21; a* = 0.14; b* = 1.99). The colour

parameters (L*, a*, and b*) were accessed by CIELAB system and the total colour difference between raw and cooked samples was calculated according to Equation (1).

$$\Delta E^* = (\Delta L^* + \Delta a^* + \Delta b^*)^{1/2}. \quad (1)$$

The measurements were conducted at 20 ± 1 °C and replicated at least eight times.

2.4. Physicochemical Analysis of Pasta

Moisture content was determined gravimetrically on raw, cooked, and lyophilised samples using an automatic moisture analyser PMB 202 (aeADAM, Milton Keynes, UK) at 130 °C, until constant weight.

Ash content was determined gravimetrically by incineration at 550 °C in a muffle furnace (Snol LHM01, Utena, Lithuania). Protein analysis was performed by the Kjeldhal method according to the ISO 20483 [31] for cereal and pulses. The determined total nitrogen content was multiplied by a conversion factor of 5.95 [32] to obtain the pasta crude protein content.

The total lipids content was determined according to the procedure used for cereals and derived products in the Portuguese standard method NP4168 [33], as described in Batista et al. [34].

Soluble, insoluble, and total dietary fibre contents of *Laminaria ochroleuca* and all pasta samples (raw and cooked) were evaluated according to AOAC 991.43 [35] with the specific modifications for *Psyllium* fibre suggested by Lee et al. [36].

Minerals presence and content were carried out by inductively coupled plasma (ICP) spectrometry (iCAP Spectrometer equipped with ASX-520 AutoSampler(Thermo Scientific, Waltham, MA, USA)). Briefly, 0.25 g of lyophilized sample was weighed, transferred to digestion vessels, and 9 mL of HCl and 3 mL of HNO_3 were added. The digestion (SCP Science, DigiPREP MS, Baie d'Urfe, QC, Canada) took place at 30 min/40 °C, 30 min/80 °C, and 90 min/105 °C. After cooling, distilled water was added up to 50 mL, and the solution was left to decant. Finally, the clear supernatant was used in ICP analysis. Eleven elements (Na, K, Ca, Mg, P, S, Fe, Cu, Zn, Mn, I) were determined in each sample, and were selected on the basis of their significance in the study of GF food. All chemical composition analyses were repeated at least in triplicate.

2.5. Phytochemicals and Antioxidant Activity Measurements

Raw and cooked pasta samples were subjected to extraction (in duplicates) according to Sant'Anna et al. [37]. Briefly, 1 g of lyophilized sample was mixed with 50 mL of a mixture of ethanol/water (50:50), incubated at 60 °C for 1 h under magnetic stirring, and then filtrated with Whatman filter paper n.1. The liquid extracts were recovered and used for total antioxidant activity (AA) and total phenolic content (TPC) measurements.

All the following spectrophotometric methods were performed in triplicate in a Unicam UV4 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

ABTS radical (2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate)) scavenging was performed according to Re et al. [38]. A mixture of $ABTS^+$ working solution (3 mL) and the liquid extracts (30 µL) described above were incubated at 30 °C for 6 min (A_{sample}). The absorbance (A) was measured at 734 nm, and the percentual absorbance reduction regarding the initial value (% RSA) was determined according to Equation (2).

$$RSA\ (\%) = (A_{Blank} - (A_{sample} - A_{control}))/A_{Blank} \times 100. \quad (2)$$

Two blank assays, one without sample (A_{Blank}: phosphate buffered saline buffer) and another without reagents ($A_{control}$) were performed simultaneously.

A calibration curve with Trolox aqueous solutions (0.1–1 mM) was made, and the results were expressed as millimole TEAC (Trolox equivalent antioxidant capacity) per gram of sample (dry basis).

The antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was also measured. In this case, 50 µL of each liquid extract was mixed with 2 mL of the DPPH radical working solution (6×10^{-5} M), and was incubated, in the dark, at room temperature for 16 min. The absorbance of the samples, control, and blank was measured at 515 nm. Results were expressed as described for the ABTS procedure.

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method. Briefly, 250 µL of sample, 1875 µL of deionised water, 125 µL Folin–Ciocalteu (1 M), and 250 µL of Na_2CO_3 (10%, *w/v*) were mixed and incubated for 1 h in the dark at room temperature. Absorbance was measured at 765 nm against water. Gallic acid (20–120 mg/L) was used as a standard for quantification, and the results were expressed as milligram GAE (gallic acid equivalent) per gram of sample (dry basis).

2.6. Mechanical Characterization of the Pasta

Texture measurements were performed on raw and cooked pasta samples, in a TA-XT plus texturometer (Stable Microsystems, Godalming, United Kingdom) with a 5 kg load cell in a 20 °C controlled temperature room.

Texture profile analysis was performed on all raw pasta samples according to the method already described in Fradinho et al. [30]. Regarding cooked pasta, compression and tension tests were performed. Before each test, pasta samples were cooked in boiling water during optimal cooking time, rinsed with distilled water, and drained. Three types of texture measurements were performed:

- Cutting: the firmness of cooked pasta samples was measured following AACC method 66–50.01 [29]. Pasta firmness (N) was determined by measuring the cutting force required to cut three cooked tagliatelle strips using a blade set with guillotine (HDP/BSG) that cut 2.5 mm into the sample at 0.17 mm/s. From this test, adhesiveness (N·s), which is the resistance of the material when the probe is recessing, was also measured.
- Stickiness: pasta stickiness was defined as the maximum peak force required to separate the probe from the sample surface (peak height), and the area under the peak represented the work of adhesion. Three tagliatelle strips were centrally aligned under a circular plexiglass probe (44 mm diameter) on a raised platform and were retained within a circular slot (48 mm diameter) made in a base plate. The samples were compressed for 2 s with an applied force of 9.807 N at 0.5 mm/s. The precision of the stickiness measurement decreased as elapsed time increased. Therefore, the time for stickiness measurements was set at 15 min after draining.
- Extensibility: cooked pasta extensibility characteristics were determined using a Kieffer Dough and Gluten Extensibility Rig (A/KIE). Sample loading and test were conducted as follows: a tagliatelle strip was placed across the grooved region on the sample plate. The hook probe was positioned under the strip and then raised upward at 2.0 mm/s, stretching the strip until rupture. From this tension test, two parameters were obtained: the maximum resistance to extension (Rmax, N) and the extensibility until rupture (ERmax, mm).

Each test was replicated at least seven times in each pasta formulation.

Dynamic oscillatory rheology measurements were used to monitor the viscoelastic characteristics of raw and cooked pasta samples. After pasta preparation, the dough was divided into two fractions: one portion was immediately tested, whereas another portion was cut into circular disks (30 mm diameter, 2 mm height) and cooked at OCT before testing. Small amplitude oscillatory shear testing was conducted at least in duplicate in a controlled stress rheometer (MARS III, Haake, Karlsruhe, Germany) using serrated parallel plate geometry (PP 20:20 mm diameter) to avoid the slip effect and 2 mm gap (previously optimized). Surface geometry was covered with paraffin oil to prevent moisture loss. Samples were rested before rheological testing to allow temperature equilibration (5 min, 20 °C—previously optimized). Then, stress sweep tests were run at 1 Hz from 0.1 to 100 Pa to assess the linear viscoelastic region (LVR). Finally, the mechanical spectra were performed through frequency sweep tests from 0.1 to 10 Hz (20 °C, 10 Pa) within the LVR previously defined for each sample.

2.7. Statistical Analysis

The experimental data was statistically analysed using RStudio (Version 1.1.463 2009–2018 RStudio, Inc., Boston, USA). Analysis of variance (one-way ANOVA) was used to assess significant differences between samples at a significance level of 95% ($p < 0.05$). Multiple comparisons were performed by Tukey HSD (honestly significant difference) est. All results are presented as average ± standard deviation (s.d.).

3. Results and Discussion

3.1. Pasta Cooking Quality

All pasta samples presented the same optimal cooking time—2 min. The results of the pasta quality performance upon cooking are shown in Figure 2.

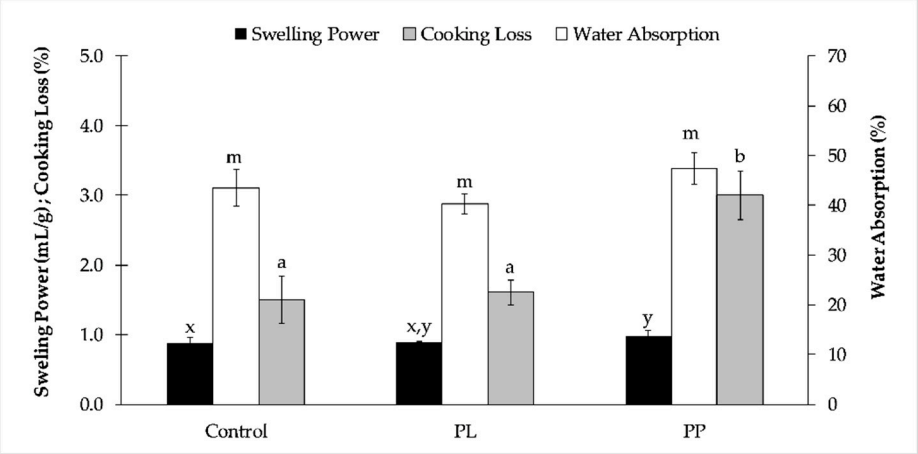


Figure 2. Cooking quality parameters of pasta prepared with *Laminaria ochroleuca* liquid extract (PL), alga purée (PP), and control (without alga). Data are presented as mean ± standard deviation (s.d.). Different letters in the same parameter correspond to significant differences ($p < 0.05$, one-way ANOVA, *post-hoc* Tukey test).

Control and *L. ochroleuca* liquid extract (PL) pastas present similar cooking behaviour. Pasta prepared with *Laminaria* purée (PP) showed significantly ($p < 0.05$) higher swelling power than the control, probably due to the alga capacity of retaining the cooking water.

Due to the lack of the gluten network, gluten-free (GF) pastas rely mostly on starch for creating the matrix and entrapping the water. Thus, GF pastas generally show higher cooking loss (CL) than wheat pastas [39]. However, all GF pastas developed here showed lower CL than durum wheat spaghetti commercial samples reported by Bonomi et al. [40], proving their quality upon cooking. The higher CL of PP could be related to the higher fibre content of this pasta (Table 1), and also with its lower *Psyllium* content—(1.5% (*w/w*) against 2% (*w/w*) of the other formulations), which has a high water absorption capacity at about 1 g *Psyllium*/15 g water [41].

3.2. Colour Stability upon Cooking

Colour stability is an important attribute when working with colourful food products. In Figure 3, the results of colour parameters obtained in raw and cooked pastas are shown.

As expected, the addition of *L. ochroleuca* changed the colour of the GF pasta, ranging from the whitish colour of control pasta, to green of PP, reflecting the use of the whole alga in the formulation. The most noticeable feature was the colour difference between raw and cooked pastas ($12.8 \leq \Delta E^* \leq 16.1$). These results mean that the thermal processing applied to the GF pastas was responsible for a significant ($p < 0.05$) modification of the sample's colour, meaning a pigment loss (leaching). However, a

lower ΔE* was observed in PP pasta (with alga purée), which could indicate the resistance of the product to the thermal procedure applied, as already observed in pasta with microalgae incorporation [42].

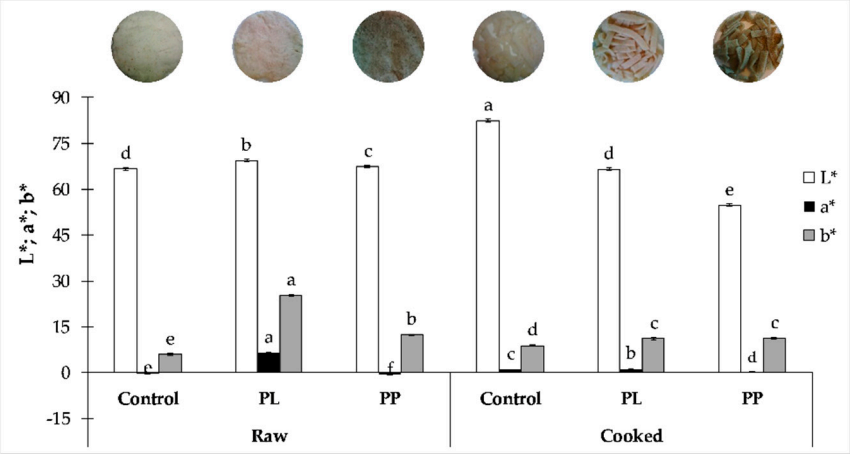


Figure 3. Colour parameters (L*, a*, b*) of raw and cooked pastas. Different letters in the same parameter (e.g., L* raw and cooked) correspond to significant differences ($p < 0.05$, one-way ANOVA, *post-hoc* Tukey test).

Regarding a* and b* colour parameters, both PL and PP showed a significant ($p < 0.05$) decrease in redness and yellowness, probably associated with pigment loss during cooking (brownish tonality of the cooking water), especially fucoxanthin and phlorotannins, major pigments of brown algae [10,43]. These results are supported by the decrease of phenolic compounds and antioxidant activity in cooked pastas (Figure 4) and the report by Prabhasankar et al. [21] for wheat pasta supplemented with *Undaria pinnatifida*.

3.3. Physicochemical Analysis of Pasta

In Table 1, the chemical composition of raw and cooked pasta samples is presented.

Table 1. Centesimal composition of raw and cooked pasta with *Laminaria* liquid extract (PL) and *Laminaria* purée (PP), and control (without alga).

			Raw Pasta			Cooked Pasta		
			Control	**PL**	**PP**	**Control**	**PL**	**PP**
Moisture		(g/100 g)	50.3 ± 0.2 ^{d}	49.2 ± 0.4 ^{d,e}	48.3 ± 0.5 ^{e}	65.4 ± 1.4 ^{b}	62.9 ± 0.9 ^{c}	67.6 ± 1.1 ^{a}
Ash		(g/100 g, d.b)	0.5 ± 0.0 ^{e}	1.3 ± 0.1 ^{b}	1.9 ± 0.1 ^{a}	0.4 ± 0.0 ^{e}	0.8 ± 0.0 ^{d}	1.1 ± 0.1 ^{c}
Lipids			2.2 ± 0.2 ^{a}	0.7 ± 0.0 ^{c}	1.7 ± 0.3 ^{a,b}	0.6 ± 0.2 ^{c}	0.9 ± 0.1 ^{c}	1.1 ± 0.1 ^{b,c}
Protein			7.7 ± 0.2 ^{c}	8.2 ± 0.2 ^{a}	8.3 ± 0.2 ^{a}	7.7 ± 0.0 ^{b,c}	8.2 ± 0.1 ^{a,b}	8.1 ± 0.0 ^{a,b,c}
Fibre	Insoluble		-	-	-	4.8 ± 0.4 ^{b}	4.4 ± 0.4 ^{b}	7.1 ± 0.1 ^{a}
	Soluble		-	-	-	0.9 ± 0.3 ^{b}	1.4 ± 0.5 ^{b}	2.4 ± 0.2 ^{a}
	Total		-	-	-	6.1 ± 0.7 ^{b}	5.6 ± 0.8 ^{b}	9.5 ± 0.3 ^{a}
Minerals	Na	(mg/100 g, d.b)	74.1 ± 2.8 ^{e}	145.7 ± 3.1 ^{c}	229.1 ± 4.8 ^{a}	70.0 ± 3.3 ^{e}	110.4 ± 2.6 ^{d}	159.4 ± 0.3 ^{b}
	K		325.3 ± 3.3 ^{e}	558.6 ± 3.7 ^{b}	761.1 ± 3.4 ^{a}	272.4 ± 4.4 ^{f}	394.4 ± 7.7 ^{d}	495.2 ± 8.8 ^{c}
	Ca		7.4 ± 1.6 ^{c}	14.6 ± 1.0 ^{b}	50.3 ± 1.9 ^{a}	9.9 ± 1.8 ^{c}	15.0 ± 0.5 ^{b}	47.3 ± 1.8 ^{a}
	Mg		28.3 ± 0.2 ^{e}	38.9 ± 0.4 ^{c}	55.1 ± 1.1 ^{a}	27.6 ± 0.5 ^{e}	34.3 ± 0.1 ^{d}	51.9 ± 0.6 ^{b}
	P		115.8 ± 2.3 ^{b}	123.2 ± 1.1 ^{a}	121.9 ± 0.5 ^{a}	109.5 ± 0.7 ^{c}	110.0 ± 1.5 ^{c}	116.9 ± 0.9 ^{b}
	S		109.0 ± 4.2 ^{c}	126.2 ± 1.6 ^{b}	144.8 ± 1.4 ^{a}	104.7 ± 1.8 ^{c}	121.3 ± 2.8 ^{b}	150.3 ± 3.0 ^{a}
	Fe		0.7 ± 0.4 ^{b}	0.5 ± 0.3 ^{b}	0.5 ± 0.2 ^{b}	1.2 ± 0.1 ^{b}	3.9 ± 0.7 ^{a}	1.5 ± 0.4 ^{b}
	Cu		0.3 ± 0.3 ^{b}	1.1 ± 0.4 ^{a,b}	1.7 ± 0.5 ^{a}	0.3 ± 0.1 ^{b}	0.4 ± 0.2 ^{b}	0.6 ± 0.0 ^{b}
	Zn		1.1 ± 0.0 ^{a}	1.6 ± 0.3 ^{a}	1.4 ± 0.3 ^{a}	1.3 ± 0.1 ^{a}	1.7 ± 0.1 ^{a}	1.6 ± 0.4 ^{a}
	Mn		0.8 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	0.9 ± 0.2 ^{a}	0.7 ± 0.1 ^{a}
	I		0.1 ± 0.0 ^{e}	0.8 ± 0.0 ^{a}	0.5 ± 0.0 ^{c}	0.1 ± 0.0 ^{e}	0.6 ± 0.0 ^{b}	0.4 ± 0.0 ^{d}

Data are presented as mean ± SD. Different letters in the same parameter (e.g., ash raw and cooked) correspond to significant differences ($p < 0.05$, one-way ANOVA, *post-hoc* Tukey test). *Laminaria ochroleuca* liquid extract (PL), *Laminaria ochroleuca* purée (PP).

Celiac disease is an auto-immune disorder characterized by malabsorption of nutrients by the small intestine, leading to multiple nutritional deficiencies [44]. A gluten-free diet is a lifelong therapy for the celiac population and gluten-sensitive individuals. However, GF foods are very often nutritionally unbalanced, providing excess calories, and being high in lipids and low in fibre content [5,45,46]. The pastas developed in the present study showed very low lipid content (as *Laminaria ochroleuca* has only about 7% (*w/w*) lipids, 55% of which were unsaturated fatty acids [47] and just 0.8% (*w/w*) of lipids for the rice flour [30]. *Laminaria* incorporation (either liquor or purée) contributed significantly ($p < 0.05$) to the protein increase in pasta. The energy value was also lower—*Laminaria* pastas showed values between 202–207 kcal/100 g (fresh weight), much lower than the energy value (369 kcal/100 g, fresh weight) reported for semolina pasta with *Undaria pinnatifida* [21], and 310–361 kcal/100 g for commercial GF dry pasta [45,48].

Laminaria addition significantly increased ($p < 0.05$) the insoluble fraction of dietary fibre. Seaweed's high fibre content is responsible for its capacity to absorb and retain water [49], and it can be used as a source of dietary fibre in bread [50] and noodles [51], also improving the mineral content. In general, seaweed can be used as a texturing and bulking agent in low-calorie foods (e.g., meat emulsions [20]).

One of the main features of *Laminaria ochroleuca* is its mineral content, mainly K, Mg, I, and Ca [19]. Although in some cases 40% of the minerals were lost during the cooking of pasta, the amount that remained after thermal processing was still high, and some of the mineral elements were not lost during cooking (Ca, S, Zn and Mn). This alga also showed 413 mg/100 g (d.b.) and 676 mg/100 g of iodine in the liquid extract obtained by autohydrolysis [19], and even if some iodine was lost during cooking, iodine presence remained strong.

Regulation (EC) 1169/2011 [52] on the provision of food information to consumers sets that 15% of the recommended daily allowance (RDA) of each mineral should be taken into consideration when deciding what constitutes a significant amount (Table 2). Also, nutrition claims are assessed on the basis of the intake of the specific nutrient in the ready-to-eat food product. All cooked pastas showed less than 0.5 g lipids/100 g, and PP pasta had more than 3.0 g/100g of fibre content. Therefore, according to the regulation (EC) No. 1924/2006 [53] amended by regulation (EU) no. 1047/2012 [54] about the nutrition claims, all pastas can bear a claim as being "fat-free", and PP pasta could also have a claim as a "source of fibre" for products containing at least 3 g dietary fibre/100 g product, which is an added value in terms of commercialization of these *Laminaria* pastas.

Table 2. Mineral content of cooked pasta with *Laminaria* liquid extract (PL) and *Laminaria* purée (PP), and control (without alga). The % values in the columns indicate the recommended daily allowance (RDA) correspondent to the mineral content.

Minerals (mg/100 g)	Control	% $RDA_{Control}$	PL	% RDA_{PL}	PP	% RDA_{PP}
K	94.3	4.7	146.3	7.3	160.4	8.0
Ca	3.4	0.4	5.6	0.7	15.3	1.9
Mg	9.5	2.5	12.7	3.4	16.8	4.5
P	37.9	5.4	40.8	5.8	37.9	5.4
Fe	0.4	3.0	1.4	10.3	0.5	3.5
Cu	0.1	10.4	0.1	14.8	0.2	19.4
Zn	0.4	4.5	0.6	6.3	0.5	5.2
Mn	0.3	13.8	0.3	16.7	0.2	11.3
I	0.0	15.0	0.2	155.6	0.1	92.2

As the impact of the continued ingestion of nutritionally unbalanced GF foods on the health of consumers is countless, contributing to the development of associated diseases, such as chronic constipation, type 1 diabetes, thyroid malfunction, anaemia, and overweight [55–58], these pastas are a breakthrough in terms of specially designed foods for this target population.

Commercial GF cereal foods, made of refined flours or starches, are also of lower nutritional value compared to their wheat counterparts [59]. Considering the average concentrations of some

mineral elements found in the present study and a daily amount of 100 g of pasta, one can conclude that it provides more for the recommended daily allowance (RDA) of minerals than GF pasta without *Laminaria*.

As mentioned earlier, the most deficient minerals in a GF diet are Ca, Fe, Mg, and Zn. In a review, Rondanelli and co-workers [60] gathered suggestions and guidelines for supplementation with several minerals and Theethira et al. [55] reported a reduction in calcium intake by about 76%–88% of patients adhering to a GF diet. Although *Laminaria ochroleuca* is a good source of Ca, the PP pasta only contributed to 1.9% of RDA for calcium, whereas Ca supplementation in the range of 1200–1500 mg/day is recommended. The importance of calcium and vitamin D should also be taken into account considering the prevalence of metabolic bone diseases in the celiac population, and some authors [9] reported the presence of vitamin D in *Laminaria ochroleuca*, which is determinant for Ca absorption.

Iron is an essential trace element, being mostly bound to hemoglobin in circulating erythrocytes, and the amount of iron in the body depends mainly on its absorption [61]. Iron deficiency is very common in celiac disease patients, leading to anaemia, which affects up to 80% of newly diagnosed celiac patients, and pasta with *Laminaria* purée caused a significant ($p < 0.05$) increase in iron content (Table 1), equivalent to 10.3% RDA.

Zinc deficiency affects about 50% of the celiac population and can affect protein synthesis leading to growth arrest [44]; thus, a zinc supplementation between 25–40 mg/day is recommended [60]. In the present study, *Laminaria* incorporation in pasta led to an increase of Zn from 4.8% to 6.3% RDA. As GF products are usually lower in magnesium, some celiac patients need additional magnesium supplement of 200–300 mg/day in the form of magnesium oxide or magnesium chloride, whereas others can improve magnesium levels through dietary means [60]. *Laminaria* incorporation in GF pasta rendered an increase from 2.5% to 4.5% RDA of Mg, promoting an additional source of Mg to the diet.

Laminaria incorporation in pasta led also to the increase in manganese (13.8% to 16.7% RDA) and copper (10.4% to 19.4% RDA) contents. Manganese is an essential trace elemental needed for normal function and regeneration of the central nervous system and is linked to osteoporosis, epilepsy, and altered lipid and carbohydrate metabolism [62]. Regarding copper, *Laminaria* incorporation in GF pasta almost doubled its content compared to control pasta (Table 2), much higher than the values reported by Orecchio et al. [59] for commercial rice noodles and pasta. This mineral is needed for adequate growth, cardiovascular integrity, lung elasticity, neovascularization, neuroendocrine function, and iron metabolism [63].

Laminaria ochroleuca is an excellent source of iodine, and therefore an alternative to fulfilling the daily iodine needs of consumers (0.15 mg I/day), and especially in celiac sufferers, as they are more susceptible to thyroid disease than non-celiacs [56]. However, one should be aware that PL pasta would provide 155% of the daily recommended amount of iodine. Although iodine ingestion above the recommended threshold is generally well-tolerated, certain susceptible individuals, including those with pre-existing thyroid disease and the elderly, risk developing iodine-induced thyroid dysfunction [64].

3.4. Effect of Laminaria Processing on the Presence of Phytochemicals in the Pasta

The total phenolic content (TPC) and in vitro antioxidant activity (AA) of pastas was performed by DPPH and ABTS methods (Figure 4). Moreover, considering that these foods are consumed after thermal processing, and that cooking induces great changes in texture, molecule structure, content and availability [23], determinations were repeated also on cooked samples.

An earlier research study [28] showed that *Laminaria ochroleuca* and its autohydrolysis liquid extract had considerable high phlorotannin content. These compounds were marine polyphenols only produced by brown algae [65]. In this sense, the total phenolic content of all pasta samples were determined, including the control.

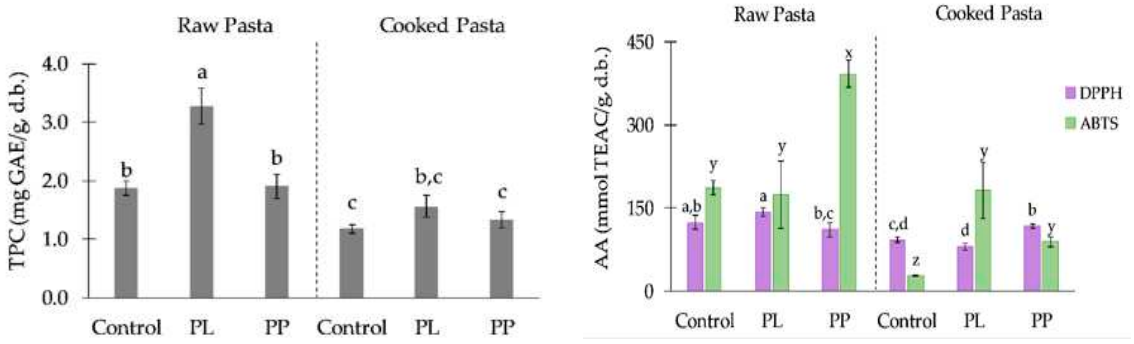


Figure 4. Total phenolic content (**a**) and antioxidant activity (**b**) of raw and cooked pastas: with *L. ochroleuca* liquid extract (PL), alga purée (PP), and control (without alga). Different letters in the same parameter correspond to significant differences ($p < 0.05$, one-way ANOVA, *post-hoc* Tukey test). DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate), TPC: total phenolic content, GAE: gallic acid equivalent, AA: antioxidant activity, TEAC: Trolox equivalent antioxidant capacity.

The TPC of raw pastas ranged from 1.87–3.28 mg GAE/g, with the control showing similar TPC than PP pasta, much higher than the values reported by Prabhasankar et al. [21] for wheat pasta with *Undaria pinnatifida* (0.56 ± 0.02 mg GAE/g). Besides the differences in extraction methods used in both studies, the main issue was the raw materials that composed the control pasta, which was produced with rice flour and *Psyllium* husk, both known for having phenolics [66,67]. The higher TPC present in PL pasta was due to the processing of the alga (autohydrolysis) prior to pasta making. Phenolic compounds are usually present in bound states as conjugates with sugars, fatty acids, or proteins. During autohydrolysis of *L. ochroleuca*, the hydro-soluble phenolic compounds were released into the liquor, becoming more available for extraction and quantification after incorporation in pasta. According to Rajauria et al. [68], the increase in phenolic compound content after hydrothermal treatment, due to the disassociation of the complexes, followed by some polymerisation of the phenolics, may be responsible for the increased antioxidant capacity. However, during cooking of pasta, part of the phenolics were leached into the cooking water, explaining the decrease in TPC and AA values. Prabhasankar et al. [69] also notes that phenolics, in the cooked control semolina pasta, were not significantly different from samples supplemented with *Sargassum marginatum.* However, in some cases, phenolics in cereal products are reported to increase with cooking, as the process can soften the hard structure and break cellular components, allowing an easier extraction from the matrix [23,48].

Regarding the antioxidant activity of raw pastas, both scavenging activity methods showed different trends. Each of the methods provided an estimate of the capacity that was dependent upon reaction time, method used, and the complexity of the reaction kinetics. On the other hand, interaction/polymerization of phenolic compounds may cause antioxidant capacity to be underestimated in fruit samples [70]. Scavenging activity of seaweed-incorporated pasta varied from 111-391 mmol TEAC/g, correspondent to 2.8%–5.2% RSA (radical scavenging activity), meaning that all samples showed weak antiradical effect. These results are similar to the ones reported for semolina pasta with *Undaria pinnatifida* [21], with fermented dough [71], and also for GF pasta with green mussel [72]. The higher AA (ABTS method) observed in PP pasta could be due to other phytochemicals rather than only phenolic compounds. Fucoxanthin was identified as the predominant pigment in brown seaweed, including *L. ochroleuca* [10]. Fucoxanthin, β-carotene, and violaxanthin are carotenoids found in seaweed that exhibit powerful antioxidant properties [73], which could explain the higher results of PP pasta. Another hypothesis to explain the increase in antioxidant features could be the presence of hydrolysed protein [74]. Unlike other carotenoids, which are commonly used as food

colorants, fucoxanthin is not sold as a bulk food ingredient [75]. Its instability due to oxidation and high extraction costs could be an opportunity for the development of *Laminaria*-enriched products.

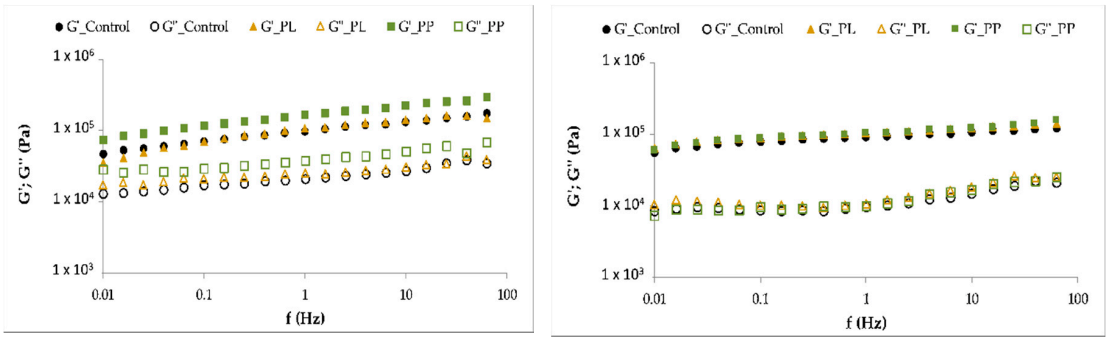
3.5. Mechanical Properties of Pasta

3.5.1. Rheology Characterization of Pasta Samples

The results from the small amplitude dynamic rheology measurements (Figure 5) of the raw and cooked pastas were expressed in terms of storage (G′) and loss (G″) moduli.

For both raw and cooked pastas, the storage modulus values (G′) were higher than those of the loss modulus (G″), which revealed the elastic nature predominance in the studied samples. Similar rheology behaviour was identified by other authors on gluten-free formulations made with rice flour [76]. Raw pastas showed a rheology behaviour slightly dependent on the frequency, especially at higher frequency values, and no differences were found between control and pastas with *Laminaria* addition (PL, PP).

After cooking, all pastas became more structured and stable, evidenced by the less frequency dependence of the viscoelastic moduli (Figure 5b). They also presented similar rheology behaviour with a minimum in G″, which indicated a second degree of structuring due to the entanglement of the biomolecules that comprise the food matrix [77]. Microstructure studies [69] revealed that the incorporation of seaweed *(Sargassum marginatum)* enhances the gluten network of pasta up to 2.5%, which resulted in improved pasta quality. Although PP pasta also had 2.5% (*w/w*) of seaweed incorporation, a different trend was observed. Unlike gluten-based foods, the cooked structure of GF foods (pasta included) is mainly due to starch physical modifications caused by the gelatinization process, forming a continuous phase of solubilized amylose and/or amylopectin [76]. Besides the positive role of starch on pasta structuring, we should also consider the role played by fibre, which is reported to disrupt the protein matrix in durum wheat pasta and noodles [78,79], and is consistent with rheology behaviour of pastas upon cooking (Figure 5).



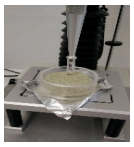
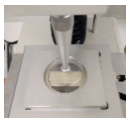
(a) (b)

Figure 5. Mechanical spectra of raw (**a**) and cooked (**b**) gluten-free (GF) pastas: with *L. ochroleuca* liquid extract (PL), alga purée (PP), and control (without alga). Closed symbols—storage modulus, G′; open symbols—loss modulus, G″.

3.5.2. Texture Properties of Pasta Samples

The texture properties of the pasta samples were evaluated before and after cooking, by both penetration and tension tests (Table 3).

Table 3. Texture parameters of raw and cooked pasta.

			Control	PL	PP
Raw Pasta	Firmness (N)		3.2 ± 0.5 ^{b}	3.6 ± 0.6 ^{b}	5.8 ± 0.4 ^{a}
	Adhesiveness (N·s)		−0.2 ± 0.1 ^{a}	−0.5 ± 0.1 ^{b}	−0.5 ± 0.1 ^{b}
	Cohesiveness		0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.3 ± 0.0 ^{b}
Cooked Pasta	Firmness (N)		2.8 ± 0.3 ^{a}	2.4 ± 0.3 ^{b}	2.3 ± 0.3 ^{b}
	Stickiness (N)		1.5 ± 0.4 ^{a}	0.7 ± 0.2 ^{b}	1.4 ± 0.4 ^{a}
	Rmax (N)		0.6 ± 0.1 ^{a,b}	0.7 ± 0.1 ^{a}	0.6 ± 0.1 ^{b}
	ERmax (mm)		4.6 ± 1.4 ^{a}	5.6 ± 1.3 ^{b}	5.5 ± 1.1 ^{b}

Data are presented as mean ± SD. Different superscript letters in the same parameter correspond to significant differences ($p < 0.05$, one-way ANOVA, *post-hoc* Tukey test).

In raw pasta, *Laminaria* addition contributed to a significant ($p < 0.05$) increase in PP firmness value, probably related to the high fibre content of this pasta. After cooking, the firmness decreased to values in the range of 2.25–2.76 N, similar to GF pasta with green mussel (1.30–2.01 N [72]). Raw PP was the less cohesive pasta, which explained the significant higher cooking loss (3%) when compared to the other samples. PP presented the highest firmness value of the analysed raw pastas, and the results were consistent with rheology measurements.

Adhesiveness increase in *Laminaria*-supplemented pastas (PL and PP) could be related to the increase in fibre content as reported by Bouasla et al. [80] for GF pasta with lentils, as fibre absorbs water and can become adhesive. However, after cooking, they showed very low stickiness values, especially PL pasta, probably resulting from a different conformation on the hydrocolloids caused by heat processing.

Laminaria addition caused a significant ($p < 0.05$) increase of pasta extensibility, which could be related to the synergy between alga biopolymers and *Psyllium*, already found in *Laminaria–Psyllium* gels [28].

3.6. Limitations and Future Work

Although the present study was not focused on the impact of incorporation of *L. ochroleuca* on the sensory quality of pasta, an informal sensory trial was performed. The consumers tested the product and stated that the taste was good (even with no salt added), and that the characteristic alga odour was less intense after cooking.

Studies on the bioavailability of the interesting compounds, namely polyphenols, minerals, and fibre, should be undertaken to evaluate the real functionally of the developed products. Also, in order to ensure consumer safety, the gluten content of pasta samples must be assessed.

4. Conclusions

This study intended to develop a GF fresh pasta that fulfils some of the requirements needed by the celiac population, and at the same time, adds value to local an under-exploited raw materials (*Laminaria ochroleuca* and rice flour from broken grains).

Laminaria ochroleuca showed promising potential to be valued, either in full or using its liquid extract, for pasta supplementation in terms of fibre and mineral contents. The GF pasta developed could bear nutrition claims for "source of fibre" and "fat-free" and showed interesting mechanical properties.

Author Contributions: P.F., M.D.T., and A.R. conceived and planned the experiments. P.F. and M.D.T. participated in sample preparation and analysis. P.F. performed the data analysis, interpretation of the results, and wrote the paper draft. I.S., A.R., and H.D. supervised the research work, contributed to the discussion of the data, and revised the manuscript.

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References

1. Khan, R.; Grigor, J.; Winger, R.; Win, A. Functional food product development-opportunities and challenges for food manufacturers. *Trends Food Sci. Technol.* **2013**, *30*, 27–37. [CrossRef]
2. Küster-Boluda, I.; Vidal-Capilla, I. Consumer attitudes in the election of functional foods. *Span. J. Mark-ESIC* **2017**, *21*, 65–79. [CrossRef]
3. Santeramo, F.G.; Carluccib, D.; De Devitiisa, B.; Secciaa, A.; Stasia, A.; Viscecchiaa, R.; Nardone, G. Emerging trends in European food, diets and food industry. *Food Res. Int.* **2018**, *104*, 39–47. [CrossRef] [PubMed]
4. Hung, Y.; Verbeke, W. Consumer evaluation, use and health relevance of health claims in the European Union. *Food Q. Prefer.* **2019**, *74*, 88–99. [CrossRef]
5. Vici, G.; Belli, L.; Biondi, M.; Polzonetti, V. Gluten free diet and nutrient deficiencies: A review. *Clin. Nutr.* **2016**, *35*, 1236–1241. [CrossRef] [PubMed]
6. Gobbetti, M.; Pontonio, E.; Filannino, P.; Rizzello, C.G.; De Angelis, M.; Di Cagnoa, R. How to improve the gluten-free diet: The state of the art from a food science perspective. *Food Res. Int.* **2018**, *11*, 22–32. [CrossRef]
7. Nascimento, A.B.; Fiates, G.M.R.; dos Anjos, A.; Teixeira, E. Gluten-free is not enough—Perception and suggestions of celiac consumers. *Int. J. Food Sci. Nutr.* **2014**, *65*, 394–398. [CrossRef]
8. Sánchez-Machado, D.I.; López-Cervantes, J.; López-Hernández, J.; Paseiro-Losada, P. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.* **2004**, *85*, 439–444. [CrossRef]
9. Kim, S.K.; Bhatnagar, I. Properties of Medicinal Food-*Laminaria*. In *Marine Medicinal Foods—Implications and Applications, Macro and Microalgae*; Kim, S.K., Ed.; Academic Press: Oxford, UK, 2011; Volume 64, pp. 85–96, doi:10.1016/B978-0-12-387669-0.00007-7.Food-Laminaria. In *Marine Medicinal Foods—Implications and Applications, Macro and Microalgae*; Kim, S.K., Ed.; Academic Press: Oxford, UK, 2011; Volume 64, pp. 85–96. [CrossRef]
10. Fernandes, F.; Barbosa, M.; Oliveira, A.P.; Azevedo, I.C.; Sousa-Pinto, I.; Valentão, P.; Andrade, P.B. The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. *J. Appl. Phycol.* **2016**, *28*, 3689–3696. [CrossRef]
11. Flórez, N.; Gonzalez-Muñoz, M.J.; Ribeiro, D.; Fernandes, E.; Dominguez, H.; Freitas, M. Algae Polysaccharides' Chemical Characterization and their Role in the Inflammatory Process. *Curr. Med. Chem.* **2017**, *24*, 149–175. [CrossRef]
12. Parada, J.; Pérez-Correa, J.R.; Pérez-Jiménez, J. Design of low glycemic response foods using polyphenols from seaweed. *J. Func. Foods* **2018**, *56*, 33–39. [CrossRef]
13. Cofrades, S.; Serdaroğlu, M.; Jiménez-Colmenero, F. Design of Healthier Foods and Beverages Containing Whole Algae. In *Functional Ingredients from Algae for Foods and Nutraceuticals*; Domínguez, H., Ed.; Woodhead Publishing: Cambridge, UK, 2013; Volume 256, pp. 609–633. [CrossRef]
14. Andrade, P.B.; Barbosa, M.; Matos, R.P.; Lopes, G.; Vinholes, J.; Mouga, T.; Valentão, P. Valuable compounds in macroalgae extracts. *Food Chem.* **2013**, *138*, 1819–1828. [CrossRef] [PubMed]

15. Roohinejad, S.; Kouba, M.; Barba, F.J.; Saljoughian, S.; Amid, M.; Greiner, R. Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Res. Int.* **2017**, *99*, 1066–1083. [CrossRef] [PubMed]
16. Bouga, M.; Combet, E. Emergence of Seaweed and Seaweed-Containing Foods in the UK: Focus on Labeling, Iodine Content, Toxicity and Nutrition. *Foods* **2015**, *4*, 240–253. [CrossRef]
17. Marfaing, H. Qualités nutritionnelles des algues, leur présent et futur sur la scène alimentaire. *Cah Nutr. Diet* **2017**, *52*, 257–268. [CrossRef]
18. Smale, D.A.; Wernberg, T.; Yunnie, A.L.E.; Vance, T. The rise of *Laminaria ochroleuca* in the Western English Channel (UK) and comparisons with its competitor and assemblage dominant *Laminaria hyperborea*. *Mar Ecol.* **2015**, *36*, 1033–1044. [CrossRef]
19. Fradinho, P.; Flórez-Fernández, N.; Sousa, I.; Raymundo, A.; Domínguez, H.; Torres Pérez, M.D. Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties. *J. Appl. Phycol.* **2019**. [CrossRef]
20. Cofrades, S.; López-López, I.; Solas, M.T.; Bravo, L.; Jiménez-Colmenero, F. Influence of different types and proportions of added edible seaweeds on characteristics of low-salt gel/emulsion meat systems. *Meat Sci.* **2008**, *79*, 767–776. [CrossRef]
21. Prabhasankar, P.; Ganesan, P.; Bhaskar, N.; Hirose, A.; Stephen, N.; Gowda, L.R.; Hosokawa, M.; Miyashita, K. Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chem.* **2009**, *115*, 501–508. [CrossRef]
22. Del Olmo, A.; Picon, A.; Nuñez, M. Cheese supplementation with five species of edible seaweeds: Effect on microbiota, antioxidant activity, colour, texture and sensory characteristics. *Int. Dairy J.* **2018**, *84*, 36–45. [CrossRef]
23. Palermo, M.A.; Pellegrini, N.; Fogliano, V. The effect of cooking on the phytochemical content of vegetables. *J. Sci. Food Agric.* **2014**, *94*, 1057–1070. [CrossRef] [PubMed]
24. Rehman, Z.; Shah, W. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chem.* **2005**, *91*, 327–331. [CrossRef]
25. Hager, A.S.; Czerny, M.; Bez, J.; Zannini, E.; Arendt, E.K. Starch properties, in vitro digestibility and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour. *J. Cereal Sci.* **2013**, *58*, 156–163. [CrossRef]
26. Palavecino, P.M.; Ribotta, P.D.; León, A.E.; Bustos, M.C. Gluten-free sorghum pasta: Starch digestibility and antioxidant capacity compared with commercial products. *J. Sci. Food Agric.* **2019**, *99*, 1351–1357. [CrossRef] [PubMed]
27. Soares, R.O. Estudos de Incorporação de Microalgas em Massa Alimentícia Isenta de Glúten. Master's Thesis, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal, 2018. Available online: https://www.repository.utl.pt/handle/10400.5/17818 (accessed on 8 October 2019).
28. Fradinho, P.; Raymundo, A.; Sousa, I.; Domínguez, H.; Torres Pérez, M.D. *Psyllium* and *Laminaria* partnership—An overview of possible food gel applications. *Appl. Sci.* **2019**, *9*, 4356. [CrossRef]
29. AACC International. *Approved Methods of Analysis*, 11th ed.; Method 66–50.01. Pasta and Noodle Cooking Quality—Firmness; AACC International: St. Paul, MN, USA, 2000. [CrossRef]
30. Fradinho, P.; Sousa, I.; Raymundo, A. Functional and thermorheological properties of rice flour gels for gluten-free pasta applications. *Int. J. Food Sci. Tech.* **2019**, *54*, 1109–1120. [CrossRef]
31. ISO 20483. *Cereals and Pulses—Determination of the Nitrogen Content and Calculation of the Crude Protein Content—Kjeldahl Method*; International Organization for Standardization: Geneva, Switzerland, 2006.
32. FAO. *Food Energy—Methods of Analysis and Conversion Factors*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2003; p. 93.
33. Norma Portuguesa NP 4168. *Cereais e Derivados—Determinação do Teor de Matéria Gorda Total*; Instituto Português da Qualidade: Lisboa, Portugal, 1991.
34. Batista, A.P.; Niccolai, A.; Fradinho, P.; Fragoso, S.; Bursic, I.; Rodolfi, L.; Biondi, N.; Tredici, M.R.; Sousa, I.; Raymundo, A. Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. *Algal. Res.* **2017**, *26*, 161–171. [CrossRef]
35. AOAC. Method 991.43. Total, Soluble, and Insoluble Dietary Fiber in Foods, 4th Revision. In *AOAC Official Methods of Analysis*, 16th ed.; AOAC International: Gaithersburg, MD, USA, 1998.

36. Lee, S.C.; Rodriguez, F.; Storey, M. Determination of soluble and insoluble dietary fiber in *Psyllium*-containing cereal products. *J. AOAC Int.* **1995**, *78*, 724–729.
37. Sant'Anna, V.; Christiano, F.D.P.; Marczak, L.D.F.; Tessaro, I.C.; Thys, R.C.S. The effect of the incorporation of grape marc powder in fettuccini pasta properties. *LWT Food Sci. Technol.* **2014**, *58*, 497–501. [CrossRef]
38. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
39. Marti, A.; Pagani, M.A. What can play the role of gluten in gluten free pasta? *Trends Food Sci. Technol.* **2013**, *31*, 63–71. [CrossRef]
40. Bonomi, F.; D'Egidio, M.G.; Iametti, S.; Marengo, M.; Marti, A.; Pagani, M.A.; Ragg, E.M. Structure-quality relationship in commercial pasta: A molecular glimpse. *Food Chem.* **2012**, *135*, 348–355. [CrossRef] [PubMed]
41. Raymundo, A.; Fradinho, P.; Nunes, M.C. Effect of *Psyllium* fibre content on the textural and rheological characteristics of biscuit and biscuit dough. *Bioact. Carbohydr. Dietary Fibre.* **2014**, *3*, 96–105. [CrossRef]
42. Fradique, M.; Batista, A.P.; Nunes, M.C.; Gouveia, L.; Bandarra, N.M.; Raymundo, A. Chlorella vulgaris and *Spirulina maxima* biomass incorporation in pasta products. *J. Sci. Food Agric.* **2010**, *90*, 1656–1664. [CrossRef] [PubMed]
43. Holdt, S.L.; Kraan, S. Bioactive compounds in seaweed: Functional food applications and legislation. *J. Appl. Phycol.* **2011**, *23*, 543–597. [CrossRef]
44. Theethira, T.G.; Dennis, M.; Leffler, D.A. Nutritional consequences of celiac disease and the gluten-free diet. *Expert Rev. Gastroenterol. Hepatol.* **2014**, *8*, 123–129. [CrossRef] [PubMed]
45. Miranda, J.; Lasa, A.; Bustamante, M.A.; Churruca, I.; Simon, E. Nutritional Differences Between a Gluten-free Diet and a Diet Containing Equivalent Products with Gluten. *Plant Foods Hum. Nutr.* **2014**, *69*, 182–187. [CrossRef]
46. Allen, B.; Orfila, C. The Availability and Nutritional Adequacy of Gluten-Free Bread and Pasta. *Nutrients* **2018**, *10*, 1370. [CrossRef]
47. Otero, P.; López-Martínez, M.I.; García-Risco, M.R. Application of Pressurized Liquid Extraction (PLE) to obtain bioactive fatty acids and phenols from *Laminaria ochroleuca* collected in Galicia (NW Spain). *J. Pharm. Biomed. Anal.* **2019**, *164*, 86–92. [CrossRef]
48. Carcea, M.; Narducci, V.; Turfani, V.; Giannini, V. Polyphenols in Raw and Cooked Cereals/Pseudocereals/Legume Pasta and Couscous. *Foods* **2017**, *6*, 80. [CrossRef]
49. Gómez-Ordóñez, E.; Jiménez-Escrig, A.; Rupérez, P. Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Res. Int.* **2010**, *43*, 2289–2294. [CrossRef]
50. Goñi, I.; Valdivieso, L.; Garcia-Alonso, A. Nori seaweed consumption modifies glycemic response in healthy volunteers. *Nutr. Res.* **2000**, *20*, 1367–1375. [CrossRef]
51. Chang, H.C.; Wu, L.C. Texture and quality properties of Chinese fresh egg noodles formulated with green seaweed (*Monostroma nitidum*) powder. *J. Food Sci.* **2008**, *73*, S398–S404. [CrossRef] [PubMed]
52. European Commission. Regulation (EC) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the Provision of Food Information to Consumers. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169&from=pt (accessed on 25 September 2019).
53. European Commission. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on Nutrition and Health Claims Made on Foods. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1924&from=PT (accessed on 25 September 2019).
54. European Commission. Regulation (EU) No 1047/2012 of 8 November 2012 Amending Regulation (EC) No 1924/2006 with Regard to the List of Nutrition Claims. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R1047&from=PT (accessed on 25 September 2019).
55. Theethira, T.G.; Dennis, M. Celiac Disease and the Gluten-Free Diet: Consequences and Recommendations for Improvement. *Dig. Dis.* **2015**, *33*, 175–182. [CrossRef] [PubMed]
56. Torres, M.I.; Palomeque, T.; Lorite, P. Celiac Disease and Other Autoimmune Disorders. In *Autoimmunity—Pathogenesis, Clinical Aspects and Therapy of Specific Autoimmune Diseases*; Chatzidionysiou, K., Ed.; IntechOpen: London, UK, 2015; Volume 6, pp. 131–151. [CrossRef]
57. Lundin, K.E.A.; Alaedini, A. Non-celiac Gluten Sensitivity. *Gastrointest. Endosc. Clin. N. Am.* **2012**, *22*, 723–734. [CrossRef]
58. Henggeler, J.C.; Veríssimo, M.; Ramos, F. Non-coeliac gluten sensitivity: A review of the literature. *Trends Food Sci. Tech.* **2017**, *66*, 84–92. [CrossRef]

59. Orecchio, S.; Amorello, D.; Raso, M.; Barreca, S.; Lino, C.; Di Gaudio, F. Determination of trace elements in gluten-free food for celiac people by ICP-MS. *Microchem. J.* **2014**, *116*, 163–172. [CrossRef]
60. Rondanelli, M.; Faliva, M.A.; Gasparri, C.; Peroni, G.; Naso, M.; Picciotto, G.; Riva, A.; Nichetti, M.; Infantino, N.; Alalwan, T.A.; et al. Micronutrients Dietary Supplementation Advices for Celiac Patients on Long-Term Gluten-Free Diet with Good Compliance: A Review. *Medicina* **2019**, *55*, 337. [CrossRef]
61. Lieu, P.T.; Heiskala, M.; Peterson, P.A.; Yang, Y. The roles of iron in health and disease. *Mol. Asp. Med.* **2001**, *22*, 1–87. [CrossRef]
62. Aschner, J.L.; Aschner, M. Nutritional aspects of manganese homeostasis. *Mol. Asp. Med.* **2005**, *26*, 353–362. [CrossRef]
63. Arredondo, M.; Núñez, M.T. Iron and copper metabolism. *Mol. Asp. Med.* **2005**, *26*, 313–327. [CrossRef] [PubMed]
64. Leung, A.M.; Braverman, L.E. Consequences of excess iodine. *Nat. Rev. Endocrinol.* **2014**, *10*, 136–142. [CrossRef] [PubMed]
65. Pereira, L. Seaweeds as Source of Bioactive Substances and Skin Care Therapy—Cosmeceuticals, Algotheraphy, and Thalassotherapy. *Cosmetics* **2018**, *5*, 68. [CrossRef]
66. Ti, H.; Li, Q.; Zhang, R.; Zhang, M.; Deng, Y.; Wei, Z.; Chi, J.; Zhang, Y. Free and bound phenolic profiles and antioxidant activity of milled fractions of different indica rice varieties cultivated in southern China. *Food Chem.* **2014**, *159*, 166–174. [CrossRef]
67. Patel, M.K.; Mishra, A.; Jha, B. Non-targeted Metabolite Profiling and Scavenging Activity Unveil the Nutraceutical Potential of *Psyllium* (*Plantago ovata Forsk*). *Front. Plant Sci.* **2016**, *7*, 1–17. [CrossRef]
68. Rajauria, G.; Jaiswal, A.K.; Abu-Ghannam, N.; Gupta, S. Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds. *Int. J. Food Sci. Technol.* **2010**, *45*, 2485–2493. [CrossRef]
69. Prabhasankar, P.; Ganesan, P.; Bhaskar, N. Influence of Indian Brown Seaweed (*Sargassum marginatum*) as an Ingredient on Quality, Biofunctional, and Microstructure Characteristics of Pasta. *Food Sci. Tech. Int.* **2009**, *15*, 0471–0479. [CrossRef]
70. Ozgen, M.; Reese, R.N.; Tulio, A.Z., Jr.; Scheerens, J.C.; Miller, A.R. Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) Methods. *J. Agric. Food Chem.* **2006**, *54*, 1151–1157. [CrossRef]
71. Fois, S.; Campus, M.; Piu, P.P.; Siliani, S.; Sanna, M.; Roggio, T.; Catzeddu, P. Fresh Pasta Manufactured with Fermented Whole Wheat Semolina: Physicochemical, Sensorial, and Nutritional Properties. *Foods* **2019**, *8*, 422. [CrossRef]
72. Vijaykrishnaraj, M.; Kumar, S.B.; Prabhasankar, P. Green mussel (*Perna canaliculus*) as a marine ingredient to enrich gluten free pasta: Product quality, microstructure and biofunctional evaluation. *J. Food Meas. Charact.* **2015**, *9*, 76–85. [CrossRef]
73. Mendis, E.; Kim, S.K. Present and Future Prospects of Seaweeds in Developing Functional Foods. In *Advances in Food and Nutrition Research*; Kim, S.K., Ed.; Academic Press: Oxford, UK, 2011; Volume 64, pp. 1–15. [CrossRef]
74. Flórez-Fernández, N.; Torres, M.D.; González-Muñoz, M.J.; Domínguez, H. Potential of intensification techniques for the extraction and depolymerization of fucoidan. *Algal. Res.* **2018**, *30*, 128–148. [CrossRef]
75. Abu-Ghannam, N.; Shannon, E. Seaweed Carotenoid, Fucoxanthin, as Functional Food. In *Microbial Functional Foods and Nutraceuticals*; Gupta, V.K., Treichel, H., Shapaval, V., Oliveira, L.A., Tuohy, M.G., Eds.; John Wiley & Sons Ltd.: Chichester, UK, 2017; Chapter 3; pp. 39–64. [CrossRef]
76. Larrosa, V.; Lorenzo, G.; Zaritzky, N.; Califano, A. Improvement of the texture and quality of cooked gluten-free pasta. *LWT Food Sci. Tech.* **2016**, *70*, 96–103. [CrossRef]
77. Ferry, J.D. *Viscoelastic Properties of Polymers*, 3rd ed.; John Wiley & Sons Inc.: Hoboken, NJ, USA, 1980; p. 672.
78. Tudorica, C.M.; Kuri, V.; Brennan, C.S. Nutritional and Physicochemical Characteristics of Dietary Fiber Enriched Pasta. *J. Agric. Food Chem.* **2002**, *50*, 347–356. [CrossRef] [PubMed]
79. Baek, J.J.; Kim, Y.; Lee, S. Functional characterization of extruded rice noodles with corn bran: Xanthophyll content and rheology. *J. Cereal Sci.* **2014**, *60*, 311–316. [CrossRef]

80. Bouasla, A.; Wojtowicz, A.; Zidoune, M.N. Gluten-free precooked rice pasta enriched with legumes flours: Physical properties, texture, sensory attributes and microstructure. *LWT Food Sci. Tech.* **2017**, *75*, 569–577. [CrossRef]

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Effect of *Arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta

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ABSTRACT

Gluten-free foods are generally nutritionally deficient and are the source of serious technological constraints. This work aimed to evaluate the technological performance of addition of two spirulina biomasses: *Arthrospira platensis* F&M-C256 and Ox Nature (resulting from different drying procedures) to gluten-free pastas, in terms of mechanical properties, antioxidant capacity, *in vitro* digestibility and sensory analysis.

Texture properties of GF pasta was not significantly ($p < 0.05$) altered by the incorporation of *A. platensis* biomass. The different drying methods applied to *A. platensis* biomasses had an impact on the bioactive compounds and the *in vitro* digestibility of the gluten-free pastas. Both *A. platensis* biomasses provided a significant ($p < 0.05$) supplementation of phenolic compounds, chlorophylls and carotenoids to the gluten-free pastas, that resulted in significantly ($p < 0.05$) higher antioxidant activity when compared to control (without *A. platensis*) and wheat pasta. Between the new gluten-free developed pastas, consumers preferred the one supplemented with 2% *A. platensis* F&M-C256 biomass. Knowing the contribution of *A. platensis* biomass addition on pasta properties is fundamental to extend the utilization of this cyanobacterium for novel foods development. These results indicate that *A. platensis* biomass is a suitable ingredient to enhance the nutritional quality of pasta, without affecting its cooking and texture quality properties, with a favourable sensory evaluation.

1. Introduction

Pasta, traditionally produced with *durum* wheat semolina, is a widely consumed food product, due to its palatability, low cost and easy preparation. Although dry pasta market represents most of the world's pasta consumption, fresh pasta market share is continuously growing, associated with the consumer's subconscious belief in the close relationship between *freshness* and *artisanal production* [1]. Fresh pasta is a recognized vehicle for food fortification (*e.g.*, fibre, vegetables, pulses and microalgae) and has received much attention from the scientific community [2,3].

Gluten-free (GF) product development presents major challenges for the food industry in terms of organoleptic, technological and nutritional characteristics. The GF food market is continuously growing, with estimated market share sales worldwide of 18% gluten-free pasta in 2022, with an annual growth rate of 7.4% [4]. Nowadays, target audience for GF foods stretches beyond coeliac sufferers. In 2015, only 9% of US gluten-free consumers followed a GF diet due to a celiac disease, while others were adopting a GF lifestyle because it made them feel healthier (12%) or wanted to lose weight (7%) [5].

In response to consumer's needs, more and more gluten-free products, such as pasta, have appeared on the market. However, these products often do not satisfy the nutritional deficiencies of these consumers in terms of dietary fibre, vitamins (B12, D), and minerals (iron, calcium, zinc) [6]. However, consumers consider GF diet hard to follow due to low availability, lack of variety, texture problems, poor palatability and high prices of the GF products [7].

Psyllium husk is a functional ingredient with a health claim approved by FDA [8] for reducing the risk of coronary heart disease. Underlying this approval is *Psyllium*'s high soluble fibre content that translates into high water absorption and gelling ability, making it possible to use *Psyllium* as a structure builder that mimics the gluten matrix [9]. Our research group has already explored *Psyllium* technological functionality in the production of biscuits [10]. In this work we intend to take advantage of both of *Psyllium*'s mentioned features in order to develop a GF functional pasta.

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Microalgae (including cyanobacteria) are generally recognized as a source of bioactive compounds and their use as nutritional supplements is becoming widely spread in western countries [11]. However, the use of microalgae biomass for incorporation in food products is still limited [12,13]. This results from the European gastronomy tradition missing this type of ingredients and also from the regulation restrictions – only a few species approved by EFSA for human consumption [14].

Microalgae comprise a vast array of bioactive compounds with known antioxidant capacity, such as carotenoids, phycobiliproteins or phenolic compounds [15]. So, microalgae have the potential to be used as natural sources of antioxidants. The therapeutic properties of TPC include anticancer, antioxidative, antibacterial, anti-allergic, anti-diabetes, anti-aging, anti-inflammatory and anti-HIV activities [16].

Arthrospira platensis (commonly known as spirulina) represents a valuable source of bioactive compounds and acts as a pigment agent. *A. platensis* is known for its high protein content, as well as γ-linolenic acid and phycocyanin contents [17]. Our research group has already successfully incorporated spirulina in wheat pasta [18] and, recently, a study with cookies showed that *A. platensis* biomass provided a significant structuring effect, in terms of texture, probably due to its high protein content (around 68% on dry biomass) [19].

Knowing the microalgae physicochemical characteristics is fundamental for the selection of the most suitable biomass to specific food technology applications and consequently successful novel foods development [20]. The content of the main biochemical components varies depending on the microalga as well as, to a large extent, on the culture conditions such as culture density, growth phase and physiological status [21,22].

Microalgae biomasses can differ greatly in their quality, colour, consistency, and nutrient content [20,23]. The different microalgae biochemical compositions can affect physical and sensorial aspects of the biomass [20,23] and of microalgae-based products such as for food gels, cookies, crackers, and pasta [3,18,19,24,25]. In particular, in the study of Fradique et al. [18] a significant difference in protein and carbohydrate contents after cooking, between pasta incorporated with *Spirulina maxima* biomass and pasta with *Chlorella vulgaris* biomass (at 2% incorporation level), was found. In a successive work, Fradique et al. [3] also showed significant differences for all the detected parameters in sensory evaluation (especially colour, depreciative fish flavour and global appreciation) between cooked fresh pasta incorporated with *Isochrysis galbana* biomass and pasta with *Diacronema vlkianum* biomass (at 2% incorporation level), confirming that the different microalgae chemical composition can strongly affect sensory aspects. In a recent work, Batista et al. [19] found a significant difference in protein and phenolic contents between cookies incorporated with *A. platensis* F&M−C256, *C. vulgaris Allma*, *Tetraselmis suecica* F&M-M33 and *Phaeodactylum tricornutum* F&M-M40 biomasses (at 6% incorporation level). Besides, *A. platensis* F&M-C256 also provided a structuring effect in terms of cookies texture. Batista et al. [25] also found that different microalgae biomasses (*A. platensis* F&M-C256, *C. vulgaris* Allma, *T. suecica* F&M-M33 and *P. tricornutum* F&M-M40, at 2% incorporation level) strongly affected sensory aspects of cookies, in particular smell, taste and global appreciation. When microalgae are integrated into foodstuffs, the aroma is an important aspect to consider. The presence of sulfuric compounds, diketones, α-ionone, and β-ionone in fresh microalga biomasses is explained by aroma formation mechanisms such as enzymatic lipid oxidation, enzymatic and chemical degradation of dimethyl sulfoniopropionate (generating dimethyl sulfide), phenylalanine (generating benzaldehyde), and carotenoids (generating ionones) [26]. Studies aimed to mitigate the taste and the smell of microalgae-based products, such as the addition of natural flavours, and to modify the colour, such as extrusion procedures, could be useful techniques to improve the appearance and the taste of these products and therefore to make them more accepted by consumers. Microalgae can also be manipulated to have high protein, high carbohydrate or high lipid content as required [27,28]. Customizing microalgae cultivation in order to obtain specific compositions can be of interest for the development of functional GF foods, in order to fulfil the needs of the target population.

The aim of this work was to develop a new *A. platensis*-based GF fresh pasta and to evaluate the addition of this cyanobacterial biomass as an innovative ingredient in order to enhance the functional properties of pasta. Two strains of *A. platensis* with different chemical composition were used in order to assess its impact on the bioactive compound's evaluation and mechanical features of the final product.

2. Materials and methods

2.1. Microalgae and other ingredients

Arthrospira platensis F&M-C256 biomass was provided by Società Agricola Serenissima (Italy). It was cultivated in GWP®-I [29] or GWP®-II photobioreactors [30,31] in semi-batch mode, in Zarrouk medium [32]. The biomass was collected by centrifugation; washed with tap water to remove excess bicarbonate; frozen (−20 °C); lyophilized; powdered and stored at −20 °C until analysis and use. For comparative purposes a spray-dried commercial biological *Arthrospira platensis* (lot O.SP-JY-170615, Ox Nature, China) was also used. *Psyllium* husk (lot 047058-02, Solgar, USA), rice flour (lot 3411/18, Ceifeira, Dacsa Atlantic, Portugal) and wheat semolina (lot 20180725 Próvida, Portugal) were purchased in local market (Table 1).

2.2. Pasta preparation

Three batches of 200 g of pasta dough were prepared, using rice flour and *Psyllium* gel in a 50/50 ratio (control). The ingredients were mixed in a food processor (Bimby TM31, Vorwerk, Wuppertal, Germany), for 3 min (speed 4) at room temperature. Then, the dough was covered in aluminium foil and allowed to equilibrate for 15 min at 25 °C in an air oven. Then, the dough was sheeted and laminated as tagliatelle (width = 6.10 mm, thickness = 2.12 mm, length = 10 cm) using a benchtop pasta machine (Atlas 150 Wellness, Marcato, Italy).

Arthrospira platensis F&M-C256 biomass and Ox Nature (from 1 to

Table 1
Biochemical composition of the both *A. platensis* used in the experiments and of *Psyllium*, rice flour and wheat semolina.

	Protein	Carbohydrate	Lipid	Ash	Total fibre	Cd	Pb	As	Hg
	(g/100 g, dry basis)					(mg/kg, wet basis)			
A. platensis F&M-C256 [19]	68.9 ± 1.0	12.8 ± 0.2	10.7 ± 0.6	6.1 ± 0.1	–	< 0.02[a]	< 0.10[a]	< 0.06[a]	< 0.1[a]
A. platensis Ox Nature	65.0 ± 2.4	17.3 ± 1.2	12.6 ± 1.4	6.5 ± 0.1	–	0.05 ± 0.01	0.35 ± 0.06	0.14 ± 0.02	< 0.1[a]
Psyllium [10]	1.6 ± 0.1	–	1.1 ± 0.0	2.6 ± 0.1	81.7 ± 6.6	–	–	–	–
Rice Flour [33]	7.9 ± 0.1	90.7	0.8 ± 0.1	0.4 ± 0.0	–	–	–	–	–
Wheat semolina[b]	12.7	73	1.1	–	3.9	–	–	–	–

Results are expressed as mean ± SD, n = 3.
* Heavy metal values below the detection threshold of the equipment.
[‡] Label information

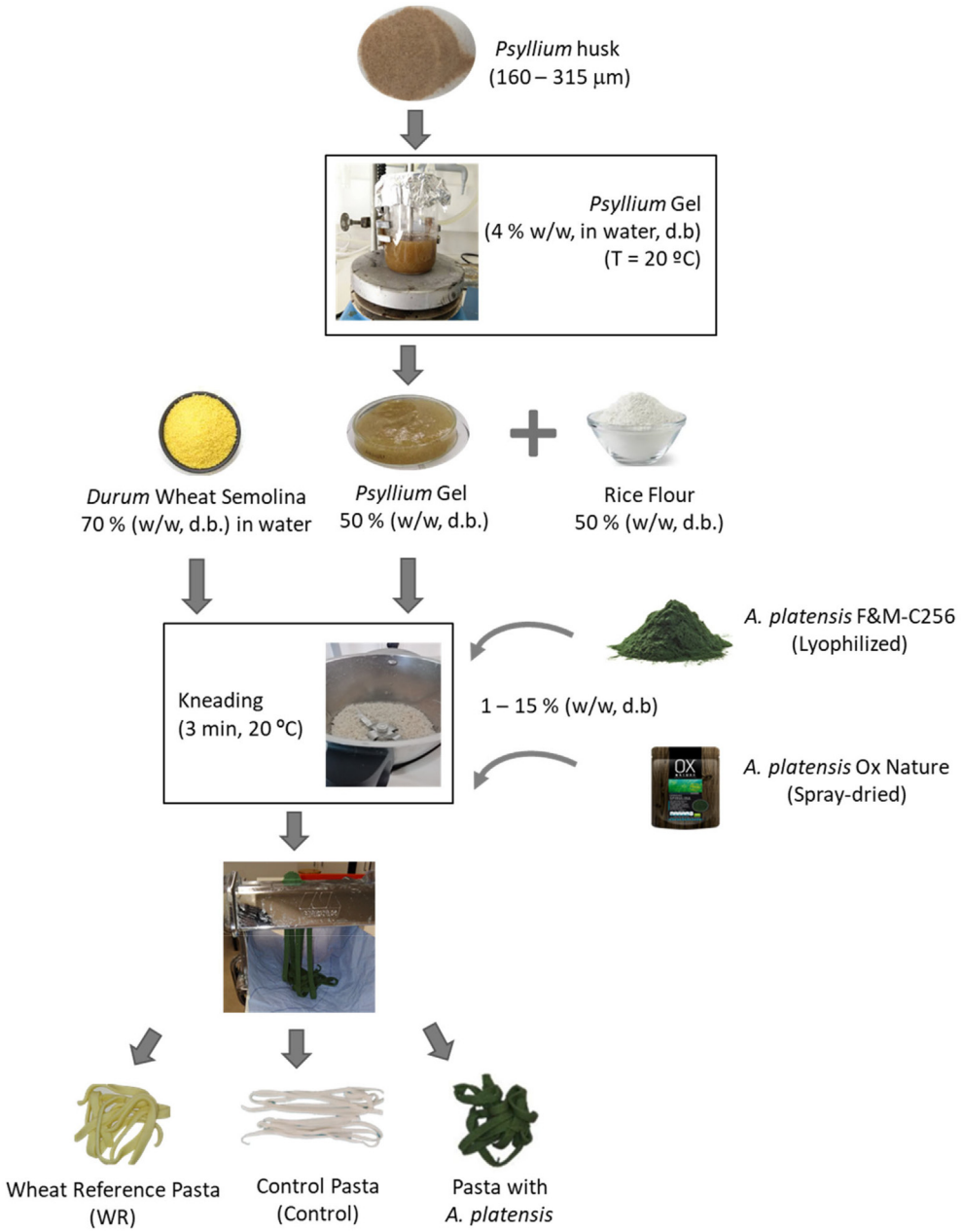

Fig. 1. Schematic representation of the experimental design for the preparation of the samples.

15% w/w) were incorporated in rice pasta dough, by replacing rice flour, in order to provide high levels of phytochemicals with bioactive activity. The incorporation limit was set by the impossibility of mixing the ingredients and properly kneading the dough. Since fresh pasta is traditionally prepared with wheat semolina, a wheat reference (WR) pasta, as described by Fradique et al. [18] with commercial *durum* semolina and water, was prepared for comparison (Fig. 1). Pasta samples were cooked at optimal cooking time, lyophilized and crushed into powder in a pestle and mortar, to be used for biochemical composition determinations, antioxidant activity and *in vitro* digestibility analyses. Physical analyses (colour, texture and rheology) were performed in the pasta preparation day.

2.3. Pasta analyses

2.3.1. Cooking quality evaluation of pasta

The tagliatelle samples were cooked in boiling distilled water for 1 min, and the cooking quality parameters were determined as reported by Fradinho et al. [33]. At least three measurements were performed for each analysis.

2.3.2. Colour analysis

The colour of raw and cooked samples was instrumentally measured using a Minolta CR-400 (Japan) colorimeter with standard illuminant D65 and a visual angle of 2°. The results were expressed according to CIELAB system colour parameters (L*, a* and b*), where L* defines lightness (values increase from 0 to 100), a* measures the degree of redness or greenness (60 to −60 positive to negative values, respectively), and b* the degree of yellowness or blueness (60 to −60 positive to negative values, respectively). The total colour difference between raw and cooked samples, was determined according to Eq. (1).

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

The measurements were conducted under the same light conditions,

using a white standard (L* = 94.61, a* = −0.53, b* = 3.62), under artificial fluorescent light at 20 ± 1 °C, replicated at least eight times for each sample.

2.3.3. Rheology measurements

After pasta dough preparation, each sample (*A. platensis* enriched; control; WR) was placed into the bottom plate of a 20 mm serrated parallel plate sensor (PP20) on the rheometer (MARS III, Haake, Karlsruhe, Germany). The temperature control was performed using an UTC-Peltier system and the gap was set at 2 mm. Edges of samples were coated with liquid paraffin, to prevent moisture losses during tests.

Stress sweep tests were conducted at 20 °C on the samples, to ensure that all measurements were carried out within the viscoelastic region. Frequency sweep tests were performed inside this region from 0.01 to 100 Hz, at 20 ± 0.5 °C. Each formulation was tested at least in triplicate. Experimental storage (elastic) - G′ and loss (viscous) - G″ moduli (Pa) data *versus* frequency - f (Hz) were fitted using power models [34], where α′, α″, b′ and b″ are the corresponding fitting parameters (Eqs. (2) and (3)).

$$G'(f) = \alpha' f^{b'} \quad (2)$$

$$G''(f) = \alpha'' f^{b''} \quad (3)$$

2.3.4. Texture analysis

Pasta texture was measured using a texturometer TA.XTplus (Stable MicroSystems, Godalming, UK) with a 5 kg load cell in a 20 °C controlled temperature room. Before each test, pasta samples were cooked in boiling water during the respective optimal cooking time, rinsed with distilled water and drained. Three types of texture measurements were preformed:

a) Cutting: the firmness of cooked pasta samples was measured following AACC method 66-50.01 [35]. Pasta firmness was determined by measuring the cutting force required to cut three cooked tagliatelle strips using a blade set with guillotine (HDP/BSG) that cut the sample at 0.17 mm/s. From this test, adhesiveness (- N.s), which is the resistance of the material when the probe is recessing, was also measured. The thickness of the samples was measured to determine the cutting distance for each sample: 1.8–2.1 mm. Each formulation was replicated at least eight times.
b) Stickiness: pasta stickiness is defined as the maximum peak force required to separate the probe from the sample surface (peak height) and the area under the peak represents the work of adhesion. To study the adhesive properties is imperative to have a procedure that forces a clean separation at the probe-material interface [36]. Three tagliatelle strips were centrally aligned under a circular plexiglass probe (44 mm diameter) on a raised platform and were retained within a circular slot (48 mm diameter) made in a base plate. The samples were compressed for 2 s with an applied force of 9.807 N at 0.5 mm/s. At least ten replicates were performed of each pasta formulation. The precision of the stickiness measurement decreases as elapsed time increases. Therefore, the time for stickiness measurements was set at 15 min after draining.
c) Extensibility: cooked pasta extensibility characteristics were determined using a Kieffer Dough & Gluten Extensibility Rig (A/KIE). Sample loading and test were conducted as follows: a tagliatelle strip was placed across the grooved region on the sample plate. The hook probe was positioned under the strip and then raised upward at 2.0 mm/s, stretching the strip until rupture. From this tension test 2 parameters were obtained: the maximum resistance to extension (Rmax, N) and the extensibility until rupture (ERmax, mm).

2.3.5. Proximate chemical composition, chlorophylls and carotenoids determination

Crude protein and lipid content were determined following Lowry et al. [37] and Marsh and Weinstein [38], respectively. Carbohydrate was determined following Dubois et al. [39]. Moisture and ash were analysed following ISTISAN protocols (ISTISAN Report 1996/34, method B, page 7 and ISTISAN Report 1996/34, pages 77–78, respectively). Chlorophylls and carotenoids were determined following Jeffrey and Humphrey [40].

Heavy metal determinations were performed in an external lab by Atomic Absorption Spectrometry with Hydride Generation (Cd and Pb), Atomic Absorption Spectrometry with Graphite Furnace (Hg), and Inductively Coupled Plasma Optical Emission Spectrometry (As).

All chemical composition analyses were repeated, at least in triplicate, and were performed on lyophilised cooked pasta.

2.3.6. Phenolics and antioxidant capacity determination

The total phenolic content assay was carried out according to Ganesan et al. [41] using the Folin Ciocalteu assay. Samples of 0.1 g of cooked pasta were dissolved in 6 mL of deionised water and sonicated for 30 min at the maximum power (frequency 20 kHz, power 130 W) maintaining the temperature below 30 °C by immerging the sample flask in an ice bath (MicrosonTM XL2000, Misonix Inc., Farmingdale, New York, USA). To 100 μL aliquots of each samples, 2 mL of 2% sodium carbonate (Sigma-Aldrich) in water were added. After 2 min, 100 μL of 50% Folin Ciocalteu reagent (Sigma-Aldrich) was added. The reaction mixture was incubated in darkness at 25 °C for 30 min. The absorbance of each sample was measured at 720 nm. Results were expressed as gallic acid equivalents (mg GAE g^{-1}) through a calibration curve with gallic acid (0 to 150 μg mL^{-1}, R^2 = 0.9907) (Sigma-Aldrich).

To evaluate the radical scavenging capacity of the cooked pasta samples, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out according to Rajauria et al. [42]. Briefly, the assay was performed in 96-well microtiter plates (Greiner Bio-One International GmbH, Germany) with 100 μL of DPPH radical solution (165 μM, in methanol, Sigma-Aldrich) and 100 μL of sample (0.2 g of lyophilised cooked pasta samples extracted for 30 min in 5 mL of a 1:5 methanol:water solution). The reaction mixtures were incubated in darkness at 30 °C for 30 min. The absorbances were measured at 517 nm using a UV–Vis spectrophotometric plate reader (BioTek USA). The antioxidant capacity of the samples was expressed in terms of μg of Vitamin C Equivalent Antioxidant Capacity (VCEAC) per gram of sample (ascorbic acid calibration curve: 0 to 10 mg mL^{-1}, R^2 = 0.992) and corresponding Radical Scavenging Activity (RSA). Two blank assays, one without sample and the other without reagents were also performed. Analyses were repeated in triplicate and performed in cooked pasta samples, previously lyophilized.

2.3.7. In vitro digestibility tests

The cooked pasta samples *in vitro* digestibility (IVD) was assessed by the Boisen and Fernández [43] method. Cooked pasta samples were weighed (1 g, particle size ≤1 mm) and transferred to 250 mL conical flasks. To each flask, phosphate buffer (25 mL, 0.1 M, pH 6.0) was added and mixed, followed by HCl (10 mL, 0.2 M) and pH was adjusted to 2.0. A freshly prepared pepsin water solution (3 mL; Applichem, Darmstadt, Germany) containing 30 mg of porcine pepsin (0.8 FIP-U/mg) was added. The flasks were incubated at 39 °C for 6 h with constant agitation (150 rpm). After, phosphate buffer (10 mL, 0.2 M, pH 6.8) and NaOH solution (5 mL, 0.6 M) were added to each sample, and pH was adjusted to 6.8. A freshly prepared pancreatin ethanol:water solution (10 mL, 50:50 v/v) containing 500 mg of porcine pancreatin (42362 FIP-U/g, Applichem, Darmstdat, Germany) was added to each sample. The flasks were incubated again at 39 °C, 150 rpm, for 18 h. A reagent blank without sample was also prepared. The undigested residues were collected by centrifugation at 18,000 ×*g* for 30 min and washed with deionised water. This procedure was repeated twice, and the final supernatant was filtered on glass-fibre membranes (47 mm Ø, pore 1.2 μm). The pellet and membranes were dried at 80 °C for 6 h, and

then at 45 °C until constant weight.

The dry matter (DM), crude protein (CP), and carbohydrate (C) *in vitro* digestibility (%) of all pasta samples was calculated from the difference between the initial biomass and the undigested biomass (after correction for the blank assay) divided by the initial biomass and multiplied by 100. Casein (Sigma Aldrich Corp., St. Louis, USA) was used as the reference material with 100% digestibility. Analyses were repeated in triplicate.

2.3.8. Sensory analysis

Sensory analysis assays were performed for gluten-free pasta with both *A. platensis* biomasses, studied at 2% incorporation level, and with the control pasta. The assays were conducted in a standardized sensory analysis room. Each pasta formulation was prepared and cooked according to the procedure described earlier and served immediately. An untrained panel of 31 individuals, 10 males and 21 females, with ages between 20 and 60, evaluated the cooked pasta samples, in terms of colour, odour, flavour, extensibility, texture and global appreciation according to 5 levels from "*very unpleasant*" to "*very pleasant*". The buying intention was also assessed, from "*would certainly buy*" to "*certainly wouldn't buy*" (5 levels). This analysis will give us the estimation of the general consumer perception and acceptation, of the newly developed pasta, against a pasta control without spirulina.

2.4. Statistical analysis

Statistical analysis of the experimental data was performed using RStudio (Version 1.1.463 – © 2009–2018 RStudio, Inc.), through variance analysis (one-way ANOVA), by the Tukey test – *Post Hoc* Comparison at a significance level of 95% ($p < 0.05$). All results are presented as mean ± standard deviation (SD).

3. Results and discussion

Samples with at least 10% microalgal biomass incorporation resulted in pasta dough with high adhesiveness and poor manufacturing abilities, which did not allow pasta lamination. Pasta dough with 4 and 5% microalgae addition was still very difficult to manufacture and had a strong fishy odour. Besides, these formulations resulted in pasta with very high cooking loss. Due to both technological and sensorial limitations, most probably due to the high content in protein, responsible for the high adhesive character and solubility in cooking, the range of *A. platensis* incorporation was set between 1 and 3% for both biomasses (F&M-C256 and Ox Nature) (Fig. 2).

3.1. Cooking quality parameters

The results obtained for the pasta cooking behaviour are presented in Fig. 3.

Cooking quality parameters are empirical determinations widely used to provide important indications about the pasta quality performance upon cooking. Pastas prepared with *A. platensis* present swelling power higher than the control. This can be explained by the capacity of the microalgae to absorb water and retain it within the starch-protein network. These results are confirmed by previous water absorption trials performed with the same *A. platensis* biomass [44], in which considerably higher water absorption (WA: 5.2 g/g_{alga}) was obtained in relation to rice flour (WA: 2.4 g/g_{flour}). Accordingly, water absorption was also affected ($p < 0.05$) by the addition of microalgae biomass, which increased from 42.8% in the control pasta to 54.9–64.5% in microalgae pastas. These values were also similar to WR water absorption. In a wheat pasta, the gluten-protein network is responsible for maintaining the pasta physical integrity during cooking, leading to generally low cooking loss (CL_{WR}: 1.45%). Although the GF pasta presented in this study has 64% less protein than WR, its structure, composed of *Psyllium* gel and rice flour, allows the reinforcement of the pasta matrix, with a significant ($p < 0.05$) cooking loss decrease (−56%), compared to WR. In a GF pasta, starch plays a decisive role as the main structuring agent, which is supported by the significant negative correlations ($p < 0.05$) between carbohydrate content and all hydration properties of cooked pastas ($r_{WA} = -0.64$, $r_{swelling} = -0.53$; $r_{CL} = -0.75$).

In the GF pastas developed, rice flour was replaced with *A. platensis*, *i.e.* mainly carbohydrates were removed and protein (+60%) and lipids (+8%) were incorporated, thus rendering a pasta with significantly ($p < 0.05$) more hydration capacity than the control. However, *A. platensis* biomasses addition negatively affected pasta cooking loss, as microalgae formulations presented values ranging from 2.08 to 3.44%. This results from the weakness of the GF matrix, leading to an easily ruptured cooked pasta structure, and thus increasing the leached solids from pasta samples into the cooking water [45]. However, there is a tendency to cooking loss reduction with higher *A. platensis* incorporation level, probably due to the protein increment in the pasta matrix. It is worth mentioning that these cooking loss values are of the same magnitude of the ones reported in other works [18,46,47] for semolina pastas enriched with this microalga, suggesting that the products obtained with *Arthrospira platensis* present high-quality in terms of cooking behaviour.

3.2. Colour stability

The results obtained for the pasta colour parameters, lightness (L*), greenness (a*) and yellowness (b*) can be seen in Table 2.

The two *A. platensis* biomasses have slightly different impact on colour, especially in chromatic parameters (a* and b*) at 3% incorporation level, which is also evident from Fig. 2.

Regarding raw pasta samples, *A. platensis* pastas present significantly ($p < 0.05$) lower lightness than control and WR pastas. Also, a reduction in all colour parameters, *i.e.* closer to the grey colour, with increasing algae concentration, can be observed. The results are similar for both *A. platensis* studied.

Regarding pasta colour stability upon cooking, all pasta samples showed significantly colour differences upon cooking ($\Delta E^* = 10.3–17$), meaning that the differences between raw and cooked samples can be detected by regular human vision [48]. These results also indicate that a certain degree of leaching could occur, but this should mainly result from degradation of colour pigments [47]. This is supported by the greenish tonality of the cooking water, which indicates some degradation of chlorophylls and phycocyanin pigments. However, by increasing *A. platensis* incorporation level (of both biomasses), a lower ΔE^* was observed which could indicate the resistance of the product to the thermal procedure applied [18]. Also, another observation is that

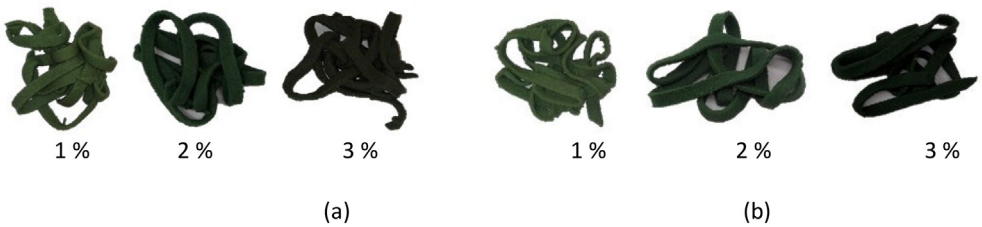

Fig. 2. Pasta dough with *A. platensis* F&M-C256 (a) and Ox Nature (b) at 1%, 2% and 3% incorporation level.

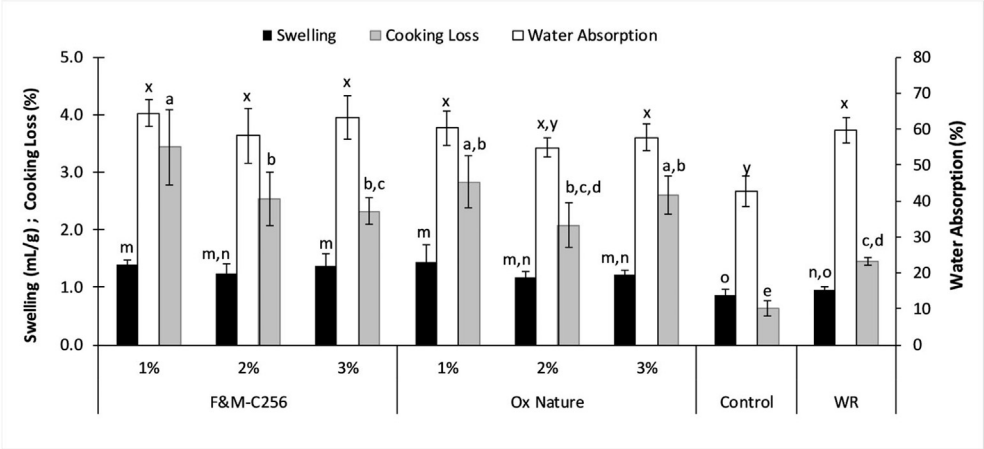

Fig. 3. Cooking quality parameters of GF pasta samples with *A. platensis* biomasses (F&M−C256, Ox Nature), control (without *A. platensis*) and wheat reference (WR). Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

chromatic parameters values, after cooking, are independent of *A. platensis* biomass concentration, suggesting that a minimum pigment leaching always occurs, probably due to a partial degradation of chlorophylls and phycocyanins. A similar phenomenon was observed in studies with gluten-free pasta enriched with legume flours [2].

Colourful eating is associated with healthy choices in food consumption [49]. Moreover, regarding thermally processed foods, such as pasta, consumers expect that a good quality pasta should maintain its colour, even after cooking [1].

To our knowledge there are no studies focusing on the supplementation of gluten-free pasta with *A. platensis*. The similarity of these results with those from wheat pasta is a clear indication of the good quality of the developed product.

3.3. Mechanical properties of dough and pasta

3.3.1. Rheology characterization of pasta dough

The results from the small amplitude dynamic rheological measurements (Fig. 4) of the uncooked pasta are expressed in terms of storage (G′) and loss (G″) moduli.

For all dough samples G′ was higher than G″, over the frequency range tested, and both moduli increased with increasing frequency. This behaviour translates into a weak gel-like network and is more noticeable in the wheat reference pasta (WR), as can be observed by the parameter b′ of the resulting power law (Table 3). When compared to the control dough, the addition of 1% microalga (and also 2% for Ox) causes the reinforcement of the dough structure (α′ increases). Since both *A. platensis* biomasses present similar biochemical composition (Table 1), the difference between supplemented pastas is probably due to the different drying method applied in *A. platensis* processing, being the commercial product more favourable in terms of restructuring.

As *A. platensis* level of incorporation continues to increase, pasta dough becomes less elastic and less stable, revealed by an increase in b′ values. As previously stated, the building of GF pasta structure is mainly achieved through gelatinization phenomena. So, only after cooking, *i.e.* after gelatinization, starch assumes the major role, resulting in a more extensible pasta compared with control (Table 4). In another study [19], the authors found that this microalga reinforced the structure of cookies in terms of texture properties.

GF doughs seem to be more structured, since viscoelastic moduli are less frequency dependent than in the case of WR. However, WR dough presents higher G′ and G″ associated with higher structured material, as corroborated by extensibility results.

3.3.2. Texture properties of cooked pasta

After cooking, the pasta texture properties were evaluated by penetration (Fig. 5) and tension tests (Table 4). The main mechanical properties in terms of quality of the cooked pasta include high firmness, high elasticity, low stickiness and surface integrity [1,50].

Firmness values of developed cooked pastas were lower (1.60 to 2.10 N), as expected, than the results reported earlier [18,46] for wheat pastas supplemented with *A. platensis*. Unlike in wheat pastas, firmness of GF pastas does not appear to be directly related with its protein content ($p < 0.05$); although there were small differences, < 1 N, the highest firmness values were observed in control pasta and in Ox_3%, which presented the lowest (3.88%) and highest protein (8.89%) values, respectively. However, Larrosa et al. [51] found that both water and protein contents strongly affected GF pasta firmness, which is probably due to the differences between pasta matrix of both studies, especially protein types [52]. Moreover, control pasta presented +28% firmness than WR (Fig. 5a), justifying the importance of carbohydrates in these products.

Table 2
Colour parameters (L*, a*, b*) of raw and cooked pasta samples with *A. platensis* biomass incorporation (F&M-C256 and Ox Nature), control (without *A. platensis*), and wheat reference (WR).

		Raw pasta			Cooked pasta		
		L*	a*	b*	L*	a*	b*
F&M-C256	1%	48.85 ± 0.83^{c}	−14.24 ± 0.21^{j,k}	10.86 ± 0.14^{g,h}	41.03 ± 5.47^{d}	−1.38 ± 0.16^{c,d}	18.81 ± 2.69^{c}
	2%	38.41 ± 0.75^{d,e}	−13.92 ± 0.23^{j}	9.86 ± 0.14^{g,h}	30.39 ± 3.08^{f}	−1.58 ± 0.32^{c,d,e}	14.24 ± 1.46^{d,e}
	3%	32.61 ± 1.01^{f}	−8.95 ± 0.40^{g}	6.71 ± 0.31^{i}	30.33 ± 2.49^{f}	−1.41 ± 0.11^{c,d,e}	15.57 ± 1.11^{d}
Ox Nature	1%	47.84 ± 0.37^{c}	−14.37 ± 0.08^{k}	11.55 ± 0.08^{f,g}	42.47 ± 2.25^{d}	−1.81 ± 0.16^{e}	18.63 ± 1.33^{c}
	2%	38.17 ± 0.70^{d,e}	−13.35 ± 0.28^{i}	10.14 ± 0.30^{g,h}	33.85 ± 2.68^{e,f}	−1.82 ± 0.22^{e}	14.57 ± 1.16^{d,e}
	3%	31.90 ± 1.34^{f}	−11.08 ± 0.37^{h}	9.03 ± 0.21^{h}	30.40 ± 2.47^{f}	−1.80 ± 0.34^{d,e}	13.33 ± 0.83^{e,f}
Control		84.22 ± 1.54^{a}	1.35 ± 0.12^{a}	10.26 ± 0.28^{g,h}	67.42 ± 5.81^{b}	0.50 ± 0.18^{b}	9.59 ± 0.77^{h}
WR		80.38 ± 1.46^{a}	−1.31 ± 0.19^{c}	28.47 ± 0.89^{a}	68.23 ± 5.61^{b}	−2.80 ± 0.29^{f}	22.31 ± 1.40^{b}

Results are expressed as mean ± SD, n = 8. Different letters in the same parameter (e.g., L* raw and cooked) show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

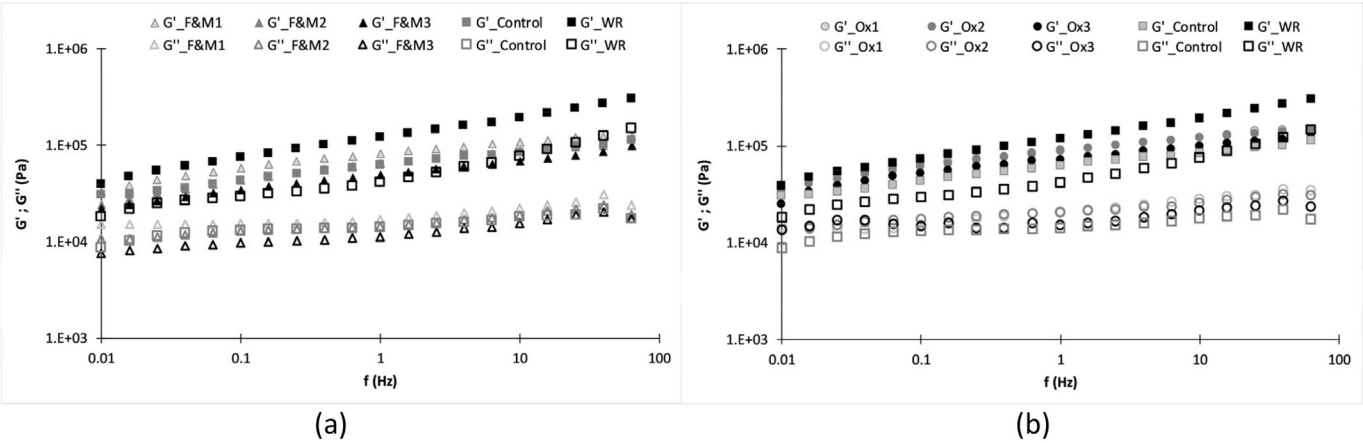

Fig. 4. Mechanical spectra of pasta dough with a) *A. platensis* F&M-C256 (1, 2, 3%), and b) *A. platensis* Ox (1, 2, 3%), control (without *A. platensis*) and wheat reference (WR). Closed symbols-G′; open symbols-G″.

Stickiness is perceived by consumers as a negative feature, being also undesirable for food packaging [36]. Stickiness values ranged from 1.76 to 2.54 N, with WR showing the highest value. *A. platensis* biomass addition did not appear to influence this texture characteristic.

Increasing the protein content of *durum* spaghetti has been shown to decrease stickiness [53]. In our study, this reported relationship was not found ($p < 0.05$), indicating once again that texture properties of GF pasta also depend on other factors than uniquely on protein content. Pasta surface stickiness is related to the amount of starch granules that exudate from the pasta matrix into the cooking water and coats the surface of the product, being a reliable indicator of cooked pasta quality [54]. Indeed, there is a positive significant correlation ($r = 0.582$, $p < 0.05$) between stickiness and cooking loss, in agreement with Larrosa et al. [51] studies on GF pastas with corn flour.

Adhesiveness values ranged between 0.02 and 0.09 -N.s and were similar to those reported by Martinez et al. [55] for commercial wheat pastas. As expected, a positive relationship between cooking loss and adhesiveness ($r = 0.647$, $p < 0.05$) was observed. A clear decrease ($p < 0.05$) in adhesiveness with the increase of *A. platensis* incorporation level was observed, indicating that this microalga contributed to the reinforcement of the internal structure of cooked pasta. Rheology results appear not to corroborate these findings, but they refer to uncooked pasta dough, *i.e.* we are comparing two different materials. The reinforcement of the structure "is visible" only after starch gelatinisation in cooked pasta.

Extensibility is an important characteristic of pasta, and in wheat-based pasta results mostly from the presence of gluten proteins, with glutenin being highly associated with the resistance to extension (Rmax) [56]. As expected, WR shows significantly ($p < 0.05$) higher extensibility parameters (resistance and distance until rupture) when compared to the GF pasta developed (Table 4). There is no correlation between pasta protein content and the extensibility parameters, reinforcing the reduction on the importance of the protein content on GF pasta, unlike gluten proteins in wheat pasta, as referred earlier.

Results of the physical parameters of dough and cooked pasta, related with the quality of the products, revealed similar behaviour regardless of *A. platensis* biomass incorporation level and origin. However, from a biochemical point of view, and based on previous works with *A. platensis* [18,19,44,57], the incorporation level will have a considerable impact on the nutritional quality of the final product. Therefore, the studies were continued with the two highest *A. platensis* concentrations (2 and 3%).

Table 4
Extensibility parameters (Rmax – resistance to extension; ERmax – Distance until rupture) of cooked pasta samples with *A. platensis* incorporation (F&M-C256 and Ox Nature), control (without *A. platensis*) and wheat reference pasta (WR).

		Rmax (N)	ERmax (mm)
F&M-C256	1%	0.51 ± 0.13^{b,c}	5.40 ± 1.12^{z}
	2%	0.40 ± 0.12^{c}	5.21 ± 1.42^{z}
	3%	0.60 ± 0.25^{b}	6.90 ± 1.89^{y,z}
Ox Nature	1%	0.53 ± 0.13^{b,c}	6.19 ± 1.27^{y,z}
	2%	0.65 ± 0.12^{b}	7.22 ± 1.57^{y}
	3%	0.65 ± 0.15^{b}	6.15 ± 1.78^{y,z}
Control		0.64 ± 0.10^{b}	6.15 ± 0.74^{y,z}
WR		1.68 ± 0.20^{a}	11.62 ± 0.85^{x}

Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

Table 3
Power law parameters (α′, α″, b′ and b″) of uncooked pasta samples with *A. platensis* (F&M-C256 and Ox Nature), control (without *A. platensis*), wheat reference (WR).

		G′		G″	
		α′	b′	α″	b″
F&M-C256	1%	80,978 ± 5215	0.14 ± 0.01	19,521 ± 1472	0.09 ± 0.01
	2%	62,567 ± 5433	0.15 ± 0.01	16,422 ± 1532	0.09 ± 0.01
	3%	49,281 ± 5529	0.16 ± 0.01	12,553 ± 2315	0.10 ± 0.02
Ox Nature	1%	91,714 ± 12,396	0.15 ± 0.01	23,007 ± 2855	0.12 ± 0.03
	2%	82,027 ± 6989	0.16 ± 0.01	20,807 ± 1532	0.09 ± 0.01
	3%	64,044 ± 4981	0.16 ± 0.01	15,734 ± 1009	0.11 ± 0.04
Control		66,765 ± 5804	0.15 ± 0.01	16,434 ± 2373	0.08 ± 0.01
WR		119,430 ± 4305	0.23 ± 0.03	49,490 ± 3269	0.22 ± 0.02

The goodness of fitting (R2) ranged from 0.890 to 0.998. Data shown is mean ± SD, n = 3.

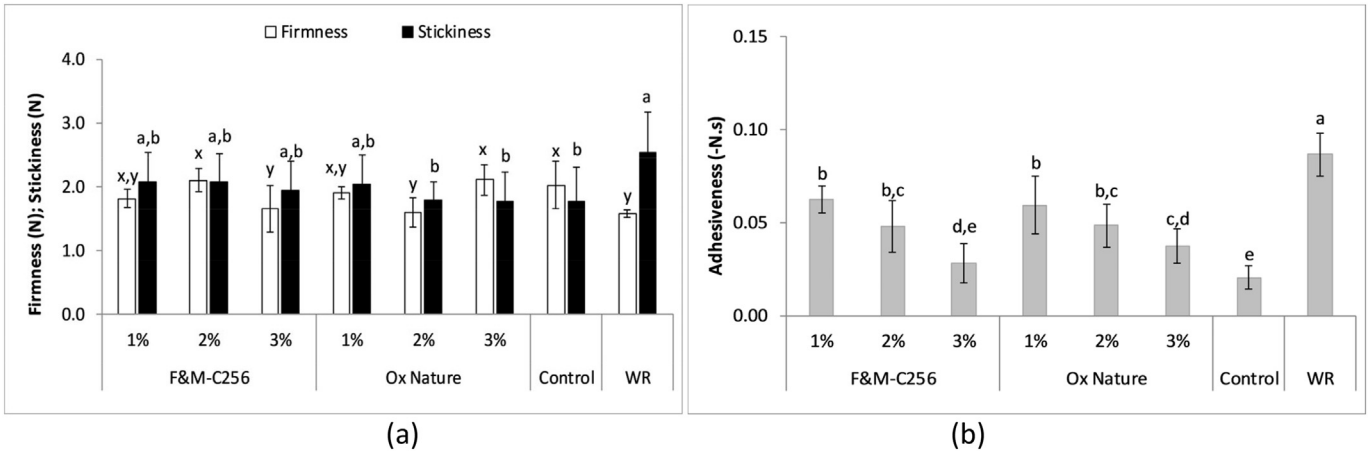

Fig. 5. Texture properties of cooked pasta samples with *A. platensis* incorporation (F&M-C256 and Ox Nature), control (without *A. platensis*) and wheat reference pasta (WR): (a) firmness and stickiness (b) adhesiveness. Data shown is mean ± SD, n = 8. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

3.4. Proximate chemical composition

Table 5 presents the proximate chemical composition of the selected cooked pasta samples.

For consumers, the main issue of GF foods is their high energy value, arising mainly from high lipid content. From proximate composition results, similar energy values (344.4–399.7 kcal/100 g, d.b.) were observed in all cooked pasta. All pasta samples presented high moisture values (59.45 to 68.46%) consistent with the results from water absorption (Fig. 3). As expected, pastas incorporated with *A. platensis* biomasses showed a significantly lower ($p < 0.05$) carbohydrate content and higher ash levels compared to control. Carbohydrates are the major component of cooked pastas, representing 93.19% (w/w) of control pasta, which is mainly composed of rice flour. With the incorporation of microalgae biomasses, rice flour is replaced by *A. platensis* biomass; consequently, the carbohydrate content of microalgae enriched pastas decreases in relation to the control sample. The incorporation of *A. platensis* biomass resulted in considerable improvements of the protein content in the final product, which results from the high concentration of these macromolecules in microalgae biomass (Table 1). Knowing the contribution of *A. platensis* biomass addition on pasta properties is fundamental to extend the utilization of this cyanobacterium and consequently successful novel foods development. Therefore, from the nutritional point of view, this is a more complex food, more interesting for diets aiming at weight control.

The presence of heavy metals in microalgae is a growing issue. As seen in Table 1, both *A. platensis* biomasses show differentiated levels of heavy metals, with *A. platensis* Ox Nature showing higher values, especially from arsenic (As) and lead (Pb). More attention should be paid to *A. platensis* cultivation conditions, due to the biosorption capacity of this cyanobacterium, in order to prevent possible heavy metals contamination. The concentration limits in plant foods according to Reg. (EC) 629/2008 [58] for lead and cadmium varied from 0.005 and 0.2 mg/kg of fresh weight and from 0.5 and 0.2 mg/kg of fresh weight, respectively. The Joint FAO/WHO Committee on Food Additives also analysed, for each heavy metal, the presumable tolerable weekly dose (PTWI), that represents the quantity of heavy metal (expressed in μg/kg of body weight in a week) that an adult man can absorb without health damages. Assuming the optimal average pasta intake is equal to 1.06 g/kg of body weight per day [59], the estimated PTWI for the four metals detected in pastas integrated with 3% *A. platensis* biomasses respects the limits gave by FAO/WHO for arsenic, cadmium, lead, and mercury (15, 7, 25 and 5 μg/kg body weight).

3.5. Bioactive compounds and antioxidant capacity

In the case of *A. platensis* pasta samples, higher amounts of TPC were found at 3% *A. platensis* incorporation level (Fig. 6a).

Pastas with *A. platensis* F&M-C256 or Ox Nature biomasses present significant ($p < 0.05$) differences in TPC contents. Since both *A. platensis* biomasses present similar biochemical composition, the different TPC contents could be due to their drying methods: *A. platensis* F&M-C256 biomass was lyophilized while *A. platensis* Ox Nature biomass was dried by spray-drying, a method that causes cell wall disruption and thus the release of significantly higher phenolic compounds than lyophilisation, as reported by Nouri and Abbasi [60]. In a previous study with *A. platensis* F&M-C256 biomass supplemented cookies, the authors reported 0.43 mg GAE/g [19], which is higher than the value obtained in the present study for GF pasta (0.24 mg GAE/g) for the same *A. platensis* biomass and incorporation level (2%). Although the matrices analysed were very different and this could have led to differences in the measured TPC values, part of this difference may also be explained

Table 5
Chemical composition and energy value of cooked pasta samples with *A. platensis* incorporation (F&M-C256 and Ox Nature), control (without *A. platensis*), and wheat reference (WR).

Sample		Protein	Lipids	Ash	Carbohydrates	Moisture	Energy value
		(% w/w, dry basis)				(% w/w)	(kcal/100 g)
F&M-C256	2%	4.0 ± 0.5^{c}	1.2 ± 0.00^{d}	0.9 ± 0.1^{b}	79.3 ± 1.5^{b}	68.2 ± 2.8^{a}	344.4
	3%	5.2 ± 1.3^{b,c}	2.2 ± 0.2^{a}	1.2 ± 0.2^{b}	82.3 ± 3.0^{b}	68.5 ± 0.9^{a}	369.8
Ox Nature	2%	6.5 ± 0.5^{b}	1.5 ± 0.1^{c}	0.8 ± 0.1^{b}	80.1 ± 5.5^{b}	66.0 ± 1.9^{a}	359.8
	3%	8.9 ± 0.7^{a}	1.9 ± 0.1^{b}	1.1 ± 0.1^{b}	80.2 ± 0.3^{b}	67.7 ± 1.4^{a}	373.5
Control		3.9 ± 1.0^{c}	1.3 ± 0.1^{c,d}	0.4 ± 0.4^{a}	93.2 ± 4.1^{a}	61.3 ± 2.6^{b}	399.7
WR		6.0 ± 1.2^{b}	1.4 ± 0.1^{c,d}	0.8 ± 0.1^{b}	83.1 ± 2.7^{b}	59.5 ± 1.0^{b}	369.3

Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA post-hoc Tukey test).

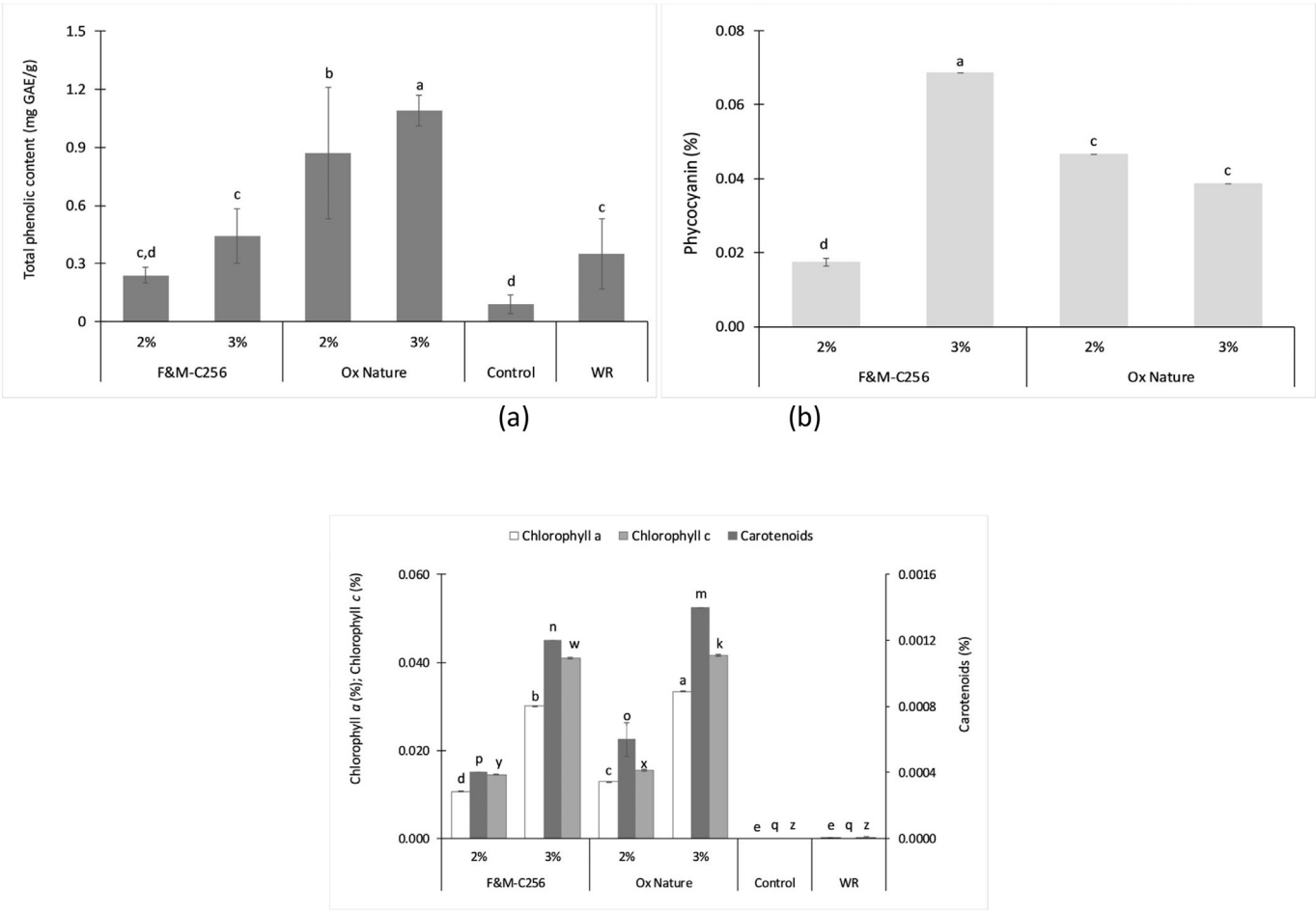

Fig. 6. (a) Total phenolic content, (b) phycocyanin content, (c) carotenoids and chlorophyll contents of cooked pasta samples with *A. platensis* (F&M−C256, Ox Nature), control (without *A. platensis*) and wheat reference (WR). Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

by the fact that TPC are hydrophilic molecules, so during pasta processing they could be leached into the cooking water.

A. platensis is also known for the presence of phycocyanin a natural blue pigment used as food colorant. It is reported that phycocyanin acts as antioxidant against free radicals, and was found to be 16 times more efficient as an antioxidant than trolox and about 20 times more efficient than ascorbic acid against haemolysis induced by peroxyl radicals in human erythrocytes [61,62].

Phycocyanin content varied between 17 and 69 mg/100 g of cooked pasta (d.b), with pasta integrated with 3% *A. platensis* F&M-C256 biomass presenting the highest value (Fig. 6b). The differences in phycocyanin content observed between pastas integrated with *A. platensis* F&M-C256 and *A. platensis* from Ox Nature could be due to different culture conditions, growth phase and physiological status adopted for the two microalgae that can influence the content of the biochemical components in algal cells [22]. A lower amount of phycocyanin when 3% *A. platensis* from Ox Nature was added in pasta compared to the 2% formulation was also observed. Considering that phycocyanin shows a high sensitivity to heat [63], it is probable that even following the same production process for pastas added with 2 and 3% *A. platensis* from Ox Nature, the manipulations adopted for pasta development and the drying procedure influenced its degradation. The two *A. platensis* biomasses presented significantly ($p < 0.05$) different amounts of these pigments, probably due to different culture conditions and drying methods.

Alves-Rodrigues and Shao [64] found carotenoids in *Chlorella* are not only an important natural food colorant and additive but also an effective stimulant of the immune response, hampering cataract and atherosclerotic development and they may reduce the risk of cardiovascular diseases and delay chronic disease. Also, microalgae chlorophylls are used as natural colorant in food and they are also known as antitumor and anti-inflammatory agent [65]. The addition of *A. platensis* biomass resulted in an effective supplementation of carotenoids and chlorophylls, which are absent in the control and WR pastas (Fig. 6c).

Gluten-free pastas present significantly ($p < 0.05$) higher scavenging activity than wheat pasta. *A. platensis* pastas show increasing antioxidant activity (DPPH and VCEAC) with increasing biomass concentration and no significant ($p < 0.05$) differences between the two biomasses was observed (Table 6).

The improvement in the presence of bioactive compounds observed in supplemented pastas led to a substantial improvement of the antioxidant activity of these pastas, as reported extensively [61]. In spite of pasta processing and cooking, *A. platensis* biomass enhanced the nutritional properties by increasing the antioxidant activity of the GF pasta as already reported by Zouari et al. [46] for *A. platensis* enriched semolina pasta. Moreover, phenolics of *A. platensis* biomass can increase both bioactivity and nutritional potential as El Gharras [66] stated for

Table 6
Antioxidant capacity of cooked pasta samples with *A. platensis* biomass (F&M−C256, Ox Nature), control (without *A. platensis*) and wheat reference (WR).

Sample		DPPH radical scavenging capacity (%)	VCEAC (μg/g)
F&M-C256	2%	56.88 ± 2.06^{b}	0.56 ± 0.04^{c}
	3%	67.97 ± 1.30^{a}	0.71 ± 0.02^{b}
Ox Nature	2%	56.02 ± 1.70^{b}	0.55 ± 0.02^{c}
	3%	70.33 ± 4.36^{a}	0.77 ± 0.02^{a}
Control		50.93 ± 2.01^{c}	0.52 ± 0.02^{d}
WR		46.62 ± 3.19^{d}	0.44 ± 0.03^{e}

Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

fruits, what also applies for microalgae.

3.6. In vitro digestibility

In the last years several studies have been conducted on the *in vitro* digestibility (IVD) of several food matrices, in order to determine the structural and chemical changes that occur in different foods under simulated gastrointestinal conditions [67]. Regarding IVD of microalgae-based foods, a previous study [19] indicated that IVD of microalgae cookies showed no significant ($p < 0.05$) differences from the control. To our knowledge, there are no studies regarding IVD in gluten-free foods supplemented with microalgae. In the present study, differences were found in dry matter IVD (Table 7). Considering the IVD of *A. platensis* F&M-C256 biomass is around 80% [19], as expected, to a higher amount of microalgal biomass correspond a decrease in digestibility. The differences between pastas with *A. platensis* F&M-C256 and *A. platensis* from Ox Nature can be related to the different biochemical composition of the two microalgal biomasses due to different culture conditions adopted during cultivation [22] that could have influenced pasta IVD. Carbohydrate digestibility was also similar for all pasta samples (Table 7), while differences emerged in protein digestibility. The control pasta is composed of *Psyllium* husk, which presents large amounts of soluble fibre (Table 1), resulting in a low protein IVD of control pasta compared to WR. Other authors reported low protein digestibility of GF pasta with hydrocolloids (19.7%) due to the network formed by them, which encapsulates the starch and avoids enzyme action [68].

In this study, protein IVD showed different trends for pastas with the two *A. platensis* biomasses. Pastas supplemented with *A. platensis* biomass from Ox Nature presented an increase in IVD, while pasta with *A. platensis* F&M-C256 biomass at 3% incorporation level slightly reduced the protein digestibility. In a study on wheat pasta with 5% *A. platensis* biomass, the authors found a higher protein IVD (71.04%) [57] compared to the protein IVD found in our study for pastas incorporated with 3% *A. platensis* biomasses. This study refers to wheat-based products, which leads to very high digestibility values when compared to those from the present study, which is supported by the higher protein IVD value obtained for WR (62.61%). Moreover, *in vitro* protein digestibility depends on the contents of phenolic compounds, polysaccharides and dietary fibre [69].

Table 7
In vitro digestibility of cooked pasta samples with *A. platensis* biomass (F&M−C256, Ox Nature), control (without *A. platensis*) and wheat reference (WR).

Sample		*In vitro* digestibility (%)		
		Dry matter	Protein	Carbohydrate
F&M-C256	2%	92.09 ± 1.03^{a,b,c}	27.97 ± 6.00^{b}	93.51 ± 0.60^{a,b}
	3%	90.84 ± 0.33^{b,c}	24.85 ± 0.66^{b}	93.08 ± 0.07^{a,b}
Ox Nature	2%	90.70 ± 1.69^{b,c}	44.98 ± 1.00^{a,b}	93.62 ± 0.50^{a,b}
	3%	89.67 ± 0.70^{c}	55.81 ± 3.47^{a,b}	92.84 ± 0.31^{b}
Control		92.95 ± 1.19^{a,b}	36.33 ± 3.07^{b}	93.99 ± 0.26^{a}
WR		94.52 ± 2.10^{a}	62.61 ± 8.98^{a}	93.84 ± 0.51^{a,b}

Data shown is mean ± SD, n = 3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test).

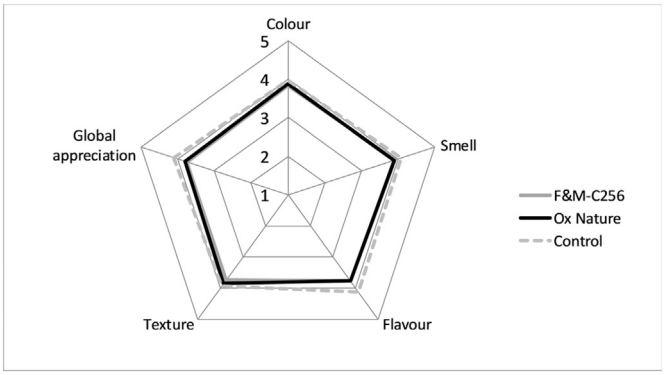

Fig. 7. Responses of the sensory analysis panel consumers (n = 31) regarding gluten-free pasta with 2% *A. platensis* F&M−C256, Ox Nature and Control. 1 – *very unpleasant*; 2 – *unpleasant*; 3 – *slightly pleasant*; 4 – *pleasant*; 5 – *very pleasant*.

3.7. Sensory evaluation

Sensory analysis was performed with cooked pasta with both biomasses (*A. platensis* F&M-C256 and Ox Nature) at 2% incorporation level, and control pasta. The selection of the optimum *A. platensis* concentration to perform this analysis was based on the bioactive and digestibility results from this work but also for comparison purposes with the results of other studies [18,46] with wheat pastas supplemented with 2% of *A. platensis*.

To our knowledge, there are no studies reporting sensory analysis of GF pasta with microalgae. From the literature reviewed concerning microalgae addition in wheat pasta, the formulations with *A. platensis* biomass seem to be the most appreciated by consumers [18].

From the average scores (Fig. 7) of the sensory parameters evaluated it is observed, although with a slight difference, that the consumer panel preferred the control sample, mainly due to the flavour component. Pastas produced with both *A. platensis* biomasses scored similarly in terms of flavour, smell and global appreciation, probably due (but not exclusively) to the similar biochemical composition of the pasta (Table 5). Earlier works [18,46] obtained similar results for wheat pasta supplement with *A. platensis* biomass, at the same level of incorporation.

Regarding texture evaluation of pastas, the consumers scored all pastas similarly (3.7–3.9), which is in agreement with the results obtained from instrumental texture. About buying intention (Fig. 8), around 68% of consumers are more prone to acquire the control pasta.

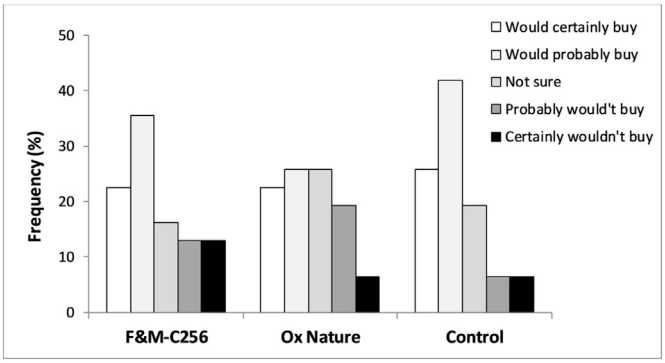

Fig. 8. Responses of the sensory analysis panel consumers regarding buying intention (n = 31) of gluten-free pasta with 2% *A. platensis* F&M−C256, Ox Nature and Control (without *A. platensis*).

Between the supplemented pastas, the formulation that includes *A. platensis* F&M-C256 biomass was the preferred, since around 58% of consumers reported that they *would buy* or *probably buy*.

4. Conclusions

This study concludes that the use of microalgae biomass, namely *Arthrospira platensis*, resulted in a product with an attractive and innovative appearance and can considerable enhance the nutritional quality of pasta, without affecting its cooking and texture quality properties, with a favourable sensory evaluation.

The differences observed in the biochemical composition of the two *A. platensis* biomasses, due to different strain specific properties and different culture conditions and processing methods, led to pastas with differentiated bioactive contents and antioxidant capacity. This feature could lead to customized applications. For both *A. platensis* biomass increasing microalgae content resulted in a significant increase in the pasta antioxidant capacity. This study suggests that spirulina-based pastas could become widely consumed GF functional foods in the future.

Declaration of competing interest

A. platensis F&M-C256 belongs to the Microalgae Culture Collection of Fotosintetica & Microbiologica S.r.l., in which M.R. Tredici and L. Rodolfi have a financial interest; all the other authors have no conflicts of interest.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

References

[1] M.A. Pagani, M. Lucisano, M. Mariotti, Traditional Italian products from wheat and other starchy flours, in: Y.H. Hui (Ed.), Handbook of Food Products Manufacturing, John Wiley & Sons Inc, New Jersey, 2007, pp. 327–388.

[2] A. Bouasla, A. Wójtowicz, M.N. Zidoune, Gluten-free precooked rice pasta enriched with legumes flours: physical properties, texture, sensory attributes and microstructure, LWT Food Sci. Technol. 75 (2017) 569–577 https://doi.org/10.1016/j.lwt.2016.10.005.

[3] M. Fradique, A.P. Batista, M.C. Nunes, L. Gouveia, N.M. Bandarra, A. Raymundo, *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source, LWT Food Sci. Technol. 50 (2013) 312–319 https://doi.org/10.1016/j.lwt.2012.05.006.

[4] D.I. Hedin, Gluten-free Globally – Important Considerations and Strategic Choices, Free From Food Expo, Euromonitor International, 2016, http://finnishfoodinnovations.fi/wp-content/uploads/2017/05/Free-from-Market-prospects_INTRO_230517_Hedin_Euromonitor.pdf , Accessed date: 4 December 2019.

[5] NSF, How much do you know about gluten? NSF International Survey, http://www.nsf.org/newsroom/nsf-survey-finds-us-consumers-struggle-to-define-identify-gluten, (2015) , Accessed date: 4 December 2019.

[6] T. Thompson, M. Dennis, L.A. Higgins, A.R. Lee, M.K. Sharrett, Gluten-free diet survey: are Americans with celiac disease consuming recommended amounts of fibre, iron, calcium and grain foods? J. Hum. Nutr. Diet. 18 (2005) 163–169 https://doi.org/10.1111/j.1365-277X.2005.00607.x.

[7] A.B. Nascimento, G.M.R. Fiates, A. dos Anjos, E. Teixeira, Gluten-free is not enough – perception and suggestions of celiac consumers, Int. J. Food Sci. Nutr. 65 (4) (2014) 394–398, https://doi.org/10.3109/09637486.2013.879286.

[8] FDA, CFR – code of federal regulations title 21, U.S. Food and Drug Administration, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.81, (2012) , Accessed date: 3 May 2019.

[9] C. Cappa, M. Lucisano, M. Mariotti, Influence of *Psyllium*, sugar beet fibre and water on gluten-free dough properties and bread quality, Carbohydr. Polym. 98 (2013) 1657–1666 https://doi.org/10.1016/j.carbpol.2013.08.007.

[10] P. Fradinho, M.C. Nunes, A. Raymundo, Biscuits enriched with *Psyllium* fibre – sensory evaluation and physicochemical comparison with market products, J. Food Sci. Technol. 52 (8) (2015) 4830–4840, https://doi.org/10.1007/s13197-014-1549-6.

[11] F.J. Barba, Microalgae and seaweeds for food applications: challenges and perspectives, Food Res. Int. 99 (2017) 969–970 https://doi.org/10.1016/j.foodres.2016.12.022.

[12] M.P. de Souza, M. Hoeltz, P.D. Gressler, L.B. Benitez, R.C.S. Schneider, Potential of microalgal bioproducts: general perspectives and main challenges, Waste Biomass Valoriz. (2018) 1–18, https://doi.org/10.1007/s12649-018-0253-6.

[13] F.J. Marti-Quijal, S. Zamuz, I. Tomašević, B. Gómez, G. Rocchetti, L. Lucini, F. Remize, F.J. Barba, J.M. Lorenzo, Influence of different sources of vegetable, whey and microalgae proteins on the physicochemical properties and amino acid profile of fresh pork sausages, LWT Food Sci. Technol. 110 (2019) 316–323 https://doi.org/10.1016/j.lwt.2019.04.097.

[14] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, Official Journal of the European Communities, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2283&qid=1551104546958&from=PT , Accessed date: 3 May 2019.

[15] H.H. Abd El-Baky, F.K. El Baz, G.S. El-Baroty, Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects, Afr. J. Biotechnol. 8 (24) (2009) 7059–7067 https://academicjournals.org/journal/AJB/article-full-text-pdf/D4B09D531593.

[16] L. Machu, L. Misurcova, J.V. Ambrozova, J. Orsavova, J. Mlcek, J. Sochor, T. Jurikova, Phenolic content and antioxidant capacity in algal food products, Molecules 20 (2015) 1118–1133, https://doi.org/10.3390/molecules20011118.

[17] A. Hongsthong, B. Bunnag, Biochemistry, biotechnology and applications, in: P.M. Gault, H.J. Marler (Eds.), Handbook on Cyanobacteria, Nova Science Publishers, New York, 2009, pp. 51–103.

[18] M. Fradique, A.P. Batista, M.C. Nunes, L. Gouveia, N.M. Bandarra, A. Raymundo, *Chlorella vulgaris* and *Spirulina maxima* biomass incorporation in pasta products, J. Sci. Food Agric. 90 (2010) 1656–1664, https://doi.org/10.1002/jsfa.3999.

[19] A.P. Batista, A. Niccolai, P. Fradinho, S. Fragoso, I. Bursic, L. Rodolfi, N. Biondi, M.R. Tredici, I. Sousa, A. Raymundo, Microalgae biomass as an alternative ingredient in cookies: sensory, physical and chemical properties, antioxidant activity and in vitro digestibility, Algal Res. 26 (2017) 161–171 https://doi.org/10.1016/j.algal.2017.07.017.

[20] A.P. Batista, L. Gouveia, N.M. Bandarra, J.M. Franco, A. Raymundo, Comparison of microalgal biomass profiles as novel functional ingredient for food products, Algal Res. 2 (2) (2013) 164–173 https://doi.org/10.1016/j.algal.2013.01.004.

[21] G. Chini Zittelli, V. Tomasello, E. Pinzani, M.R. Tredici, Outdoor cultivation of *Arthrospira platensis* during autumn and winter in temperate climates, J. Appl. Phycol. 8 (1996) 293–301 https://link.springer.com/content/pdf/10.1007%2FBF02178572.pdf.

[22] Q. Hu, Environmental effects on cell composition, in: A. Richmond, Q. Hu (Eds.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Wiley, Oxford, 2004, pp. 83–93.

[23] Â. Matos, R. Feller, E.H.S. Moecke, J.V. de Oliveira, A.F. Junior, R.B. Derner, E.S. Sant'Anna, Chemical characterization of six microalgae with potential utility for food application, J. Am. Oil Chem. Soc. 93 (2016) 963–972.

[24] A.P. Batista, M.C. Nunes, P. Fradinho, L. Gouveia, I. Sousa, A. Raymundo, J.M. Franco, Novel foods with microalgal ingredients - effect of gel setting conditions on the linear viscoelasticity of *Spirulina* and *Haematococcus* gels, J. Food Eng. 110 (2012) 182–189 https://doi.org/10.1016/j.jfoodeng.2011.05.044.

[25] A.P. Batista, A. Niccolai, I. Bursic, L. Rodolfi, N. Biondi, M.R. Tredici, I. Sousa, A. Raymundo, Microalgae biomass incorporation in salted cookies ("crackers") increases their functional properties, 4th EABA and EC Contractors' Conference and the 11th International Algae Congress, Berlin, Germany, 2017, p. 159.

[26] J. van Durme, K. Goiris, A. de Winne, L. de Cooman, K. Muylaert, Evaluation of the volatile composition and sensory properties of five species of microalgae, J. Agric. Food Chem. 61 (46) (2013) 10881–10890 https://doi.org/10.1021/jf403112k.

[27] X. Zeng, M.K. Danquah, S. Zhang, X. Zhang, M. Wu, X.D. Chen, I.-S. Nga, K. Jinga, Y. Lu, Autotrophic cultivation of *Spirulina platensis* for CO_2 fixation and phycocyanin production, Chem. Eng. J. 183 (2012) 192–197, https://doi.org/10.1016/j.cej.2011.12.062.

[28] R. Dineshkumar, R. Narendran, P. Jayasingam, P. Sampathkumar, Cultivation and chemical composition of microalgae *Chlorella vulgaris* and its antibacterial activity against human pathogens, J. Aq. Mar. Biol. 5 (3) (2017) 00119, , https://doi.org/10.15406/jamb.2017.05.00119.

[29] G. Chini Zittelli, N. Biondi, L. Rodolfi, M.R. Tredici, Photobioreactors for mass production of microalgae, in: A. Richmond, Q. Hu (Eds.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Wiley, Oxford, 2013, pp. 225–266, , https://doi.org/10.1002/9781118567166.ch13.

[30] M.R. Tredici, N. Bassi, M. Prussi, N. Biondi, L. Rodolfi, G. Chini Zittelli, G. Sampietro, Energy balance of algal biomass production in a 1-ha "Green Wall Panel" plant: how to produce algal biomass in a closed reactor achieving a high net energy ratio, Appl. Energy 154 (2015) 1103–1111 https://doi.org/10.1016/j.apenergy.2015.01.086.

[31] M.R. Tredici, L. Rodolfi, N. Biondi, N. Bassi, G. Sampietro, Techno-economic analysis of microalgal biomass production in a 1-ha Green Wall Panel (GWP®) plant, Algal Res. 19 (2016) 253–263 https://doi.org/10.1016/j.algal.2016.09.005.

[32] C. Zarrouk, Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*

(Setch et Gardner) Geitler, PhD Thesis Université de Paris, France, 1966.
[33] P. Fradinho, I. Sousa, A. Raymundo, Functional and thermorheological properties of rice flour gels for gluten-free pasta applications, Int. J. Food Sci. Technol. 54 (4) (2019) 1109–1120 https://doi.org/10.1111/ijfs.14001.
[34] A. Paraskevopoulou, V. Kiosseoglou, S. Alevisopoulos, S. Kasapis, Small deformation properties of model salad dressings prepared with reduced cholesterol egg yolk, J. Texture Stud. 28 (2) (1997) 221–237 https://doi.org/10.1111/j.1745-4603.1997.tb00112.x.
[35] AACC International, Approved methods of analysis, Method 66-50.01. Pasta and Noodle Cooking Quality—Firmness, 11th ed., AACC International, St. Paul, U.S.A, 2000, , https://doi.org/10.1094/AACCIntMethod-66-50.01.
[36] R.C. Hoseney, J. Smewing, Instrumental measurement of stickiness of doughs and other foods, J. Texture Stud. 130 (2) (1999) 123–136, https://doi.org/10.1111/j.1745-4603.1999.tb00206.x.
[37] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275 http://www.jbc.org/content/193/1/265.long.
[38] J.B. Marsh, D.B. Weinstein, Simple charring methods for determination of lipids, J. Lipid Res. 7 (1966) 574–576 http://www.jlr.org/content/7/4/574.long.
[39] M. Dubois, K.A. Gilles, J.K. Hamilton, P. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (3) (1956) 350–356 https://doi.org/10.1021/ac60111a017.
[40] S.T. Jeffrey, G.F. Humphrey, New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton, Biochem. Physiol. Pflanz. 167 (2) (1975) 191–194 https://doi.org/10.1016/S0015-3796(17)30778-3.
[41] P. Ganesan, C.S. Kumar, N. Bhaskar, Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds, Bioresour. Technol. 99 (2008) 2717–2723 https://doi.org/10.1016/j.biortech.2007.07.005.
[42] G. Rajauria, A.K. Jaiswal, N. Abu-Ghannam, S. Gupta, Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed *Himanthalia elongata* from western coast of Ireland, J. Food Biochem. 37 (2013) 322–335, https://doi.org/10.1111/j.1745-4514.2012.00663.x.
[43] S. Boisen, J.A. Fernández, Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses, Anim. Feed Sci. Technol. 68 (1997) 277–286 https://doi.org/10.1016/S0377-8401(97)00058-8.
[44] S.P. Fragoso, Desenvolvimento de bolachas com incorporação de diferentes microalgas, Master Thesis Instituto Superior de Agronomia, Universidade de Lisboa, Portugal, 2016https://www.repository.utl.pt/handle/10400.5/13367.
[45] F. Cabrera-Chávez, A.M.C. de la Barca, A.R. Islas-Rubio, A. Marti, M. Marengo, M.A. Pagani, F. Bonomi, S. Iametti, Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta, LWT Food Sci. Technol. 47 (2012) 421–426 https://doi.org/10.1016/j.lwt.2012.01.040.
[46] N. Zouari, M. Abid, N. Fakhfakh, M.A. Ayadi, L. Zorgui, M. Ayadi, H. Attia, Blue-green algae (*Arthrospira platensis*) as an ingredient in pasta: free radical scavenging activity, sensory and cooking characteristics evaluation, Int. J. Food Sci. Nutr. 62 (8) (2011) 811–813 https://doi.org/10.3109/09637486.2011.582461.
[47] G. Özyurt, L. Uslu, I. Yuvka, S. Gökdoğan, G. Atci, B. Ak, O. Işik, Evaluation of the cooking quality characteristics of pasta enriched with *Spirulina platensis*, J. Food Qual. 38 (2015) 268–272 https://doi.org/10.1111/jfq.12142.
[48] M.R. Castellar, J.M. Obón, J.A. Fernández-López, The isolation and properties of a concentrated red-purple betacyanin food colorant from Opuntia stricta fruits, J. Sci. Food Agric. 86 (2006) 122–128 https://doi.org/10.1002/jsfa.2285.
[49] L.M. König, B. Renner, Colourful = healthy? Exploring meal colour variety and its relation to food consumption, Food Qual. Prefer. 64 (2018) 66–71 https://doi.org/10.1016/j.foodqual.2017.10.011.
[50] B.A. Marchylo, J.E. Dexter, L.J. Malcolmson, Improving the texture of pasta, in: D. Kilcast (Ed.), Texture of Food, Woodhead Publishing, Ltd, Cambridge, 2004, pp. 475–500.
[51] V. Larrosa, G. Lorenzo, N. Zaritzky, A. Califano, Improvement of the texture and quality of cooked gluten-free pasta, LWT Food Sci. Technol. 70 (2016) 96–103 https://doi.org/10.1016/j.lwt.2016.02.039.
[52] A. Marti, A. Barbiroli, M. Marengo, L. Fongaro, S. Iametti, M.A. Pagani, Structuring and texturing gluten-free pasta: egg albumen or whey proteins? Eur. Food Res. Technol. 238 (2014) 217–224 https://doi.org/10.1007/s00217-013-2097-4.
[53] M.J. Sissons, N. Egan, M.C. Gianibelli, New insights into the role of gluten on durum pasta quality using reconstitution method, Cereal Chem. 82 (2005) 601–608 https://doi.org/10.1094/CC-82-0601.
[54] J.E. Dexter, R.R. Matsuo, A.W. MacGregor, Relationship of instrumental assessment of spaghetti cooking quality to the type and the amount of material rinsed from cooked spaghetti, J. Cereal Sci. 3 (1985) 39–53 https://doi.org/10.1016/S0733-5210(85)80032-1.
[55] A.S. Martinez, P.D. Ribotta, A.E. León, M.C. Añón, Physical, sensory and chemical evaluation of cooked spaghetti, J. Texture Stud. 38 (6) (2007) 666–683, https://doi.org/10.1111/j.1745-4603.2007.00119.
[56] H. Weiser, R. Kieffer, Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale, J. Cereal Sci. 34 (2001) 19–27, https://doi.org/10.1006/jcrs.2000.0385.
[57] E.R. de Marco, M.E. Steffolani, C.S. Martínez, A.E. León, Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta, LWT Food Sci. Technol. 58 (2014) 102–108, https://doi.org/10.1016/j.lwt.2014.02.054.
[58] European Commission, Commission Regulation (EC) N° 629/2008 amending Regulation (EC) N° 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Brussels, Off. J. Eur. Union 176 (2008) 6–9.
[59] G. Pounis, A. di Castelnuovo, S. Costanzo, M. Persichillo, M. Bonaccio, A. Bonanni, C. Cerletti, M.B. Donati, G. Gaetano, L. Iacoviello, Pasta consumption is negatively associated with obesity markers: an analysis of Moli-sani and INHES studies, FASEB J. 30 (1S) (2016) lb308 https://www.fasebj.org/doi/10.1096/fasebj.30.1_supplement.lb308.
[60] E. Nouri, H. Abbasi, Effects of different processing methods on phytochemical compounds and antioxidant activity of *Spirulina platensis*, Appl. Food Biotechnol. 5 (4) (2018) 221–232, https://doi.org/10.22037/afb.v%vi%i.20715.
[61] K. Chopra, M. Bishnoi, Antioxidant profile of spirulina: a blue-green microalga, in: M.E. Gershwin, A. Belay (Eds.), Spirulina in Human Nutrition and Health, CRC Press, Boca Raton, 2008, pp. 101–118.
[62] M.M. Poojary, F.J. Barba, B. Aliakbarian, F. Donsì, G. Pataro, D.A. Dias, P. Juliano, Innovative alternative technologies to extract carotenoids from microalgae and seaweeds, Mar. Drugs 14 (11) (2016) 214 https://doi.org/10.3390/md14110214.
[63] L. Jespersen, L.D. Strømdahl, K. Olsen, L.H. Skibsted, Heat and light stability of three natural blue colorants for use in confectionery and beverages, Eur. Food Res. Technol. 220 (3–4) (2005) 261–266 https://doi.org/10.1007/s00217-004-1062-7.
[64] A. Alves-Rodrigues, A. Shao, The science behind lutein, Toxicol. Lett. 150 (2004) 57–83 https://doi.org/10.1016/j.toxlet.2003.10.031.
[65] V. da Silva Ferreira, C. Sant'Anna, Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications, World J. Microbiol. Biotechnol. 33 (1) (2017) 20, https://doi.org/10.1007/s11274-016-2181-6.
[66] H. El Gharras, Polyphenols: food sources, properties and applications—a review, Int. J. Food Sci. Technol. 44 (12) (2009) 2512–2518 https://doi.org/10.1111/j.1365-2621.2009.02077.x.
[67] S.J. Hur, B.O. Lim, E.A. Decker, D.J. McClements, *In vitro* human digestion models for food applications, Food Chem. 125 (2011) 1–12 https://doi.org/10.1016/j.foodchem.2010.08.036.
[68] S. Susanna, P. Prabhasankar, A study on development of gluten free pasta and its biochemical and immunological validation, LWT Food Sci. Technol. 50 (2013) 613–621 https://doi.org/10.1016/j.lwt.2012.07.040.
[69] G.A. Annor, C. Tyl, M. Marcone, S. Ragaee, A. Marti, Why do millets have slower starch and protein digestibility than other cereals? Trends Food Sci. Technol. 66 (2017) 73–83 https://doi.org/10.1016/j.tifs.2017.05.012.

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Improving the nutritional performance of gluten-free pasta with potato peel autohydrolysis extract

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ABSTRACT

The potato processing industry produces peels, a good source of fibres, minerals and antioxidants, which could be recovered and used in the production of added-value products, such as gluten-free (GF) foods especially designed for the celiac population.

This work is focused on the application of the bioactive fraction extracted from potato peels into GF pasta. Subcritical water extraction (autohydrolysis, AH) was performed on potato peel, and the obtained AH liquid extract was characterized in terms of total phenolic content and antioxidant activity. The selected AH temperature (220 °C at 2.2 MPa) was applied to peels from Kennebec, Neiker and Agria potato varieties, and the Agria extract was selected for application in GF pasta, as this was the one with higher antioxidant activity.

The impact of Agria potato peel autohydrolysis extract on the nutritional composition and cooking quality of pasta was assessed. Results confirmed that the GF pasta enriched with potato peel extract presented suitable technological properties, coupled with attractive colour and with increased total phenolic content and antioxidant activity, which can contribute to improve the offer of GF products in the market.

1. Introduction

The development of gluten-free products with balanced nutritional quality is a trend of the food industry. Also, sustainability concerns are in todays' agenda, driving the better use of resources to the development of added-value foods (e.g. Iriondo-DeHond et al., 2019; Pal & Suresh, 2016).

Potatoes are the fourth most important food crop in the world, with an estimated production of 368 million tonnes in 2018. China is the biggest producer of potatoes worldwide, with about one third of the world's potatoes produced in China and India (FAOSTAT, 2019). Potato cultivated globally belongs to just one botanical species, *Solanum tuberosum* L., with thousands of varieties with great differences in size, shape, colour, texture, flavour and cooking characteristics (FAO, 2008).

During harvest and storage of potatoes, up to 30% are discarded due to undersized potatoes, which currently have a low added value being used primarily for animal feed (Priedniece, Spalvins, Ivanovs, Pubule, & Blumberga, 2017). Also, the world potato sector is undergoing major changes, with an increase in potato processed products such as fries, chips, mashed and canned potatoes which generate large amounts of peels and outer flesh layers. Besides, potato is the major starch source since the extraction procedure is simpler in comparison to cereal starches (Torres et al., 2020; Torres, Chenlo, & Moreira, 2018). The by-products of potatoes generated from the extensive applications in various industries represent rich sources of phenolic compounds and oligosaccharides, and their biological properties have been extensively studied (Akyol, Riciputi, Capanoglu, Caboni, & Verardo, 2016; Gientka, Aleksandrzak-Piekarczyk, Bzducha-Wróbel, Synowiec, & Błazejak, 2019; Jeddou et al., 2018). Therefore, the valorisation of potato by-products is crucial to the sustainability of the potato industry and can add value to products such as gluten-free foods, being reintroduced into the food value chain, a good bioeconomy practice.

Following the current trends in green chemistry, using water as the only extraction agent (Díaz-Reinoso, González-Muñoz, & Domínguez, 2017), the use of green technologies to extract functional components from potato peel could be an alternative to value this resource. Water-based extraction is food compatible, non-expensive, and environmentally friendly but has low selectivity with low extraction efficiency (Flórez-Fernández, Torres, González-Muñoz, & Domínguez, 2019; López-Hortas et al., 2018). To increase hydroextraction the autohydrolysis (AH) process can be performed using high temperature and pressure to boost aqueous extractions efficiency. This technology

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allows the formation of new compounds with antioxidant activity due to Maillard and caramelization reactions, and also the extraction of apolar components as a consequence of the lower water polarity (Plaza, Amigo-Benavent, del Castillo, Ibáñez, & Herrero, 2010; Rajauria, Jaiswal, Abu-Ghannam, & Gupta, 2010). The authors of the present study have expertise in the use of this technology in the recovery of bioactive-rich extracts from several matrices, namely mushrooms (Huamán-Leandro et al., 2020), seaweeds (Fradinho et al., 2020) and potato peel (Torres et al., 2020).

In this context, the development of gluten-free pasta nutritionally enriched in bioactive compounds from potato peel extract was studied, in line with the sustainable and healthy food trends.

2. Materials and methods

2.1. Materials and sample preparation

Low-size discarded potatoes from three Galician (Spain) varieties (Kennebec, Agria and Neiker) were kindly provided by INORDE (Instituto Ourensán de Desenvolvemento Económico). Rice flour (Ceifeira, Dacsa Atlantic, lot 3411/18) and *Psyllium* husk from India (Solgar, lot 107028-01, USA) were purchased in the local market. *Psyllium* was milled (Pulverisette 14 Premium, Fritsch, Germany) and sieved to 160–315 μm particles.

2.2. Subcritical water extraction and extract characterization

The potato peel coarse milled fraction (0.25–2 mm) was subjected to hydrothermal processing (autohydrolysis) with compressed hot water, in a pressurized reactor (Parr 4848, Illinois, USA), operating at around 2.2 MPa. The most abundant potato variety (Kennebec) was used for optimization of the autohydrolysis conditions: the ground peel contacted with water at a liquid:solid ratio of 15:1 (w/w), and a temperature sweep from 160 to 220 °C was performed. The conditions used for autohydrolysis were selected based on the results previously reported by Torres et al. (2020).

Then, the selected temperature (220 °C) was applied to the other potato varieties, namely Agria and Neiker. The liquid fraction was separated by filtration and it was stored at 4 °C until further analysis, within a week.

Colour determination, total phenolic content (TPC) and antioxidant activity evaluation were performed in all autohydrolysis liquid extracts as described in Sections 2.5 and 2.7.

2.3. Fresh pasta preparation and sampling

Potato peel autohydrolysis liquid extract was processed with *Psyllium* husk: 4% w/w, dry basis (d.b.) at 40 °C, 10 min to obtain a gel, at the conditions earlier described by Fradinho, Soares, Niccolai, Sousa, and Raymundo (2020). This gel was mixed with rice flour (50:50 ratio) in a thermoprocessor (Bimby TM31, Vorwerk, Wuppertal, Germany) at 25 °C for 3 min. Then, the dough was sheeted and laminated as tagliatelle using a benchtop pasta machine (Atlas 150 Wellness, Marcato, Italy), covered with aluminium foil, and placed in an air oven at 25 °C for 15 min to equilibrate the structure. A control sample (without AH extract) was also prepared using the same procedure.

All pasta samples (uncooked and cooked) were lyophilized (Scanvac Coolsafe 55-4, Labogene, Allerød, Denmark), crushed into powder and stored at 20 °C in a desiccator until further chemical analysis. Physical analyses (colour, texture and rheology) were performed immediately after preparation.

2.4. Cooking quality parameters

Pasta was cooked for 2 min as previously optimized by the authors (Fradinho, Raymundo, Sousa, Domínguez, & Torres, 2019) and water absorption, swelling power and cooking loss parameters were assessed following the AACC method 66-50 (AACC, 1999a) procedure already described in Fradinho, Sousa, and Raymundo (2019). Each determination was performed at least three times.

2.5. Colour measurements

The colour measurements of autohydrolysis extracts, and raw and cooked pasta samples were performed instrumentally using a CR-400 colorimeter (Minolta, Japan) with standard illuminant D65 and a visual angle of 2°. The colour parameters (L*, a* and b*) were accessed by CIELAB system, where L* defines lightness, and a* (degree of redness/greenness) and b* (degree of yellowness/blueness) are the chromaticity parameters. The colour stability upon cooking was determined by the total colour difference between raw and cooked pasta samples ($\Delta E^*_{raw} - \Delta E^*_{cooked}$) and between potato enriched pasta (PPE) and the control ($\Delta E^*_{control} - \Delta E^*_{PPE}$), according to Eq. (1).

$$\Delta E^* = [(L^*_{raw} - L^*_{cooked})^2 + (a^*_{raw} - a^*_{cooked^2}) + (b^*_{raw} - b^*_{cooked})^2]^{1/2} \quad (1)$$

The measurements were conducted under the same light conditions, using a white standard (L* = 94.61, a* = −0.53, b* = 3.62), under artificial fluorescent light at 20 ± 1 °C and replicated at least 6 times.

2.6. Nutritional composition

Raw and cooked pasta samples were analysed for their moisture content according to AACC method 44-15.02 (AACC, 1999b) and ash NP518 (1986), based on gravimetric methods. Total lipid analysis was carried out according to the Portuguese standard method NP4168 (1991) and protein content was determined by the Kjeldahl method following the (ISO 20483, 2006) using nitrogen conversion factor 5.95 (FAO, 2003) to obtain the pasta crude protein content.

Soluble, insoluble, and total dietary fibre contents of cooked pasta samples were evaluated according to AOAC 991.43 (1998) with the specific modifications for *Psyllium* fibre suggested by Lee, Rodriguez, and Storey (1995).

Mineral (Na, K, Ca, Mg, P, S, Fe, Cu, Zn, Mn, I) analysis was carried out by inductively coupled plasma (ICP) spectrometry (iCAP Spectrometer equipped with ASX-520 AutoSampler, Thermo Scientific, Waltham, MA, USA) following the procedure described in (Fradinho et al., 2019).

Carbohydrate content was determined by difference to 100% of main constituents (moisture, ash, protein and lipids). All chemical analyses were carried out at least in triplicate.

2.7. Determination of bioactive compounds

Raw and cooked pasta samples were subjected to extraction (in duplicates) following the procedure described by Sant'Anna, Christiano, Marczak, Tessaro, and Thys (2014). 1 g of lyophilized sample was mixed with 50 mL ethanol/water (50:50), incubated at 60 °C/1 h, under magnetic stirring, and filtered with Whatman filter paper n.1. The liquid extracts were recovered and used for total antioxidant activity (AA) and total phenolic content (TPC) measurements. All the following spectrophotometric methods were performed in triplicate in a Unicam UV4 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Two blank assays, one without sample and the other without reagents were also performed in each method.

2.7.1. Total phenolic content

Total phenolic content was analysed by the Folin-Ciocalteu method using gallic acid (20–120 mg/L) as standard (Singleton & Rossi, 1965). Liquid extracts (250 μL) were mixed with distilled water (1875 μL), Folin-Ciocalteu's phenol reagent (125 μL) and sodium carbonate (250 μL, 10%, w/v). Samples were incubated in darkness at 20 °C for

Table 1
Characterization of liquid extracts obtained from autohydrolysis of the three varieties of potato peels: Kennebec, Neiker and Agria.

Potato variety	Kennebec				Neiker	Agria
Final temperature (°C)	160	180	200		220	
TPC (g GAE/L)	0.34 ± 0.00^{f}	0.55 ± 0.02^{e}	0.87 ± 0.02^{d}	1.34 ± 0.01^{a}	0.93 ± 0.02^{c}	1.02 ± 0.03^{b}
Antioxidant Activity (mmol TEAC/L)						
ABTS	0.29 ± 0.02^{e}	3.14 ± 0.39^{d}	5.80 ± 0.75^{c}	9.76 ± 0.05^{b}	14.38 ± 0.85^{a}	15.68 ± 0.26^{a}
DPPH	0.54 ± 0.01^{d}	0.67 ± 0.01^{b,c}	0.70 ± 0.02^{b,c}	0.71 ± 0.01^{b}	0.66 ± 0.01^{c}	0.79 ± 0.01^{a}
Colour						
L*	33.03 ± 2.37^{a}	35.4 ± 1.72^{a}	25.42 ± 1.48^{b}	19.73 ± 1.38^{c}	20.88 ± 1.50^{c}	20.23 ± 0.58^{c}
a*	0.62 ± 0.43^{c}	0.72 ± 0.66^{c}	4.79 ± 0.61^{b}	7.70 ± 1.16^{a}	7.57 ± 0.82^{a}	6.61 ± 0.78^{a,b}
b*	1.07 ± 0.67^{b}	4.62 ± 0.90^{a}	2.40 ± 0.51^{a,b}	2.35 ± 1.68^{a,b}	2.71 ± 1.07^{a,b}	−5.13 ± 0.88^{c}

Data are presented as mean ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.001$, one-way ANOVA, *post-hoc* Tukey test). TPC: Total phenolic content; DPPH: α,α-diphenyl-b-picrylhydrazyl; ABTS: 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate); GAE: gallic acid equivalent; TEAC: Trolox Equivalent Antioxidant Capacity.

1 h, before absorbance measurements (765 nm). The results were expressed as milligram GAE (gallic acid equivalent) per gram of sample (dry basis).

2.7.2. Antioxidant activity

The ABTS radical cation ($ABTS^{\cdot+}$) [2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate)] scavenging activity was determined according to Re et al. (1999). Briefly, liquid extract (30 μL) and $ABTS^{\cdot+}$ solution (3 mL) were mixed and incubated at 30 °C for 6 min, and the absorbance was measured at 734 nm against Phosphate Buffered Saline (PBS).

The antiradical capacity against the α, α-diphenyl-b-picrylhydrazyl (DPPH) radical was also measured (Brand-Williams, Cuvelier, & Berset, 1995). Liquid extracts (75 μL) were mixed with the DPPH radical working solution (3 mL). After 16 min, the decrease in absorbance (515 nm) was measured. The percentage of absorbance reduction regarding the initial value was used to calculate the inhibition percentage.

The results obtained with both methods were expressed as mmol TEAC (Trolox Equivalent Antioxidant Capacity) per gram of sample (dry basis). A calibration curve with Trolox aqueous solutions (0.1–1 mM).

2.8. Texture analysis

Raw and cooked pasta texture parameters were determined using a texturometer TA.XTplus (Stable MicroSystems, Godalming, UK) with a 5 kg load cell in a 20 °C controlled temperature room. Each test was replicated at least eight times.

The texture profile analysis (TPA) of raw pasta samples was performed in penetration mode, using a cylindrical 10 mm acrylic probe. The dough was moulded in acrylic discs (61.5 mm diameter and 18 mm height) and rested for 15 min before the probe plunged 8 mm at 1 mm/s. From the force vs. time texturograms, the parameters which discriminate the sample's texture - firmness (N), adhesiveness (N.s) and cohesiveness, were determined.

Cooked pasta texture analysis was performed after cooking pasta strands for 2 min and stopping the cooking by rinsing pasta with distilled water. The firmness (N) and adhesiveness (-N.s) properties were assessed by a cutting test following AACC method 66-50.01 (AACC, 1999a), using a blade set with guillotine (HDP/BSG). Pasta stickiness (N) after cooking was determined by compressing (9.807 N) pasta strands at 0.5 mm/s for 2 s (Fradinho, Sousa, & Raymundo, 2019). Cooked pasta extensibility was performed using a Kieffer Dough & Gluten Extensibility Rig (A/KIE), that stretched the pasta strand at 2.0 mm/s until rupture, to obtain the maximum resistance to extension (Rmax, N) and the extensibility until rupture (ERmax, mm).

2.9. Rheology measurements

Small amplitude oscillatory shear (SAOS) testing was used to monitor the viscoelastic characteristics of raw and cooked pasta samples. After pasta preparation, the dough was divided into two fractions: one portion was immediately tested, whereas another portion was cut into circular disks (30 mm diameter, 2 mm height) and cooked before testing. SAOS measurements were conducted at least in triplicate in a controlled stress rheometer (MARS III, Haake, Karlsruhe, Germany) using serrated parallel plate geometry (PP20, 20 mm diameter) to avoid the slip effect with a 2 mm gap (previously optimized). Surface geometry was covered with paraffin oil to prevent moisture loss. Samples were rested before rheological testing to allow temperature equilibration (5 min at 20 °C, previously optimized). Stress sweep tests were run at 1 Hz from 0.1 to 100 Pa to assess the linear viscoelastic region (LVR). Then, the mechanical spectra were performed through frequency sweep tests from 0.1 to 10 Hz (20 °C, 10 Pa) within the LVR previously defined for each sample.

2.10. Statistical analysis

All above trials, namely autohydrolysis extractions, pasta formulations and physicochemical analysis, were made in triplicate. Experimental data is presented as average ± standard deviation (s.d.). Significant differences between samples were assessed by Student *t*-test or one-way ANOVA followed by Tukey's HSD test at 95% confidence level ($p < 0.05$) using RStudio (version 1.1.463 – © 2009–2018 RStudio, Inc.).

3. Results and discussion

3.1. Subcritical water extraction and extract characterization

Autohydrolysis liquid extracts were characterized in terms of colour, total phenolic content and antioxidant activity evaluated using in vitro assays DPPH and ABTS (Table 1).

Results from the autohydrolysis trials with Kennebec potato peel showed that increasing temperature from 180 to 220 °C led to a darker colour of the liquid extract, expressed in terms of the reduction of L* parameter. At higher temperatures, the extraction of hydrosoluble compounds is favoured, so there is an increase of TPC in the autohydrolysis liquid extract, as also reported by several authors (e.g. Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017; Plaza et al., 2010). In addition, the formation of Maillard and caramelization products at higher temperatures could contribute to this effect.

Potato peel is an excellent source of total phenolics as 50% of them are in the peel and adjoining tissues of potato. Moreover, around 65% of potato peel phenolics are in the free-form (Nara, Miyoshi, Honma, & Koga, 2006; Riciputi et al., 2018) and consequently are easily recovered

by relatively mild extraction procedures such as the eco-friendly autohydrolysis. In fact, Singh and Saldaña (2011) reported the use of subcritical water extraction method (i.e. autohydrolysis) to obtain phenolic acids from potato peel, namely caffeic and chlorogenic acids. These compounds are the predominant free-form phenolic acids in potato peel and account for 57% of total antioxidant activity (Nara et al., 2006). Based on these results, 220 °C was the AH temperature selected for apply to the other potato peel varieties.

Applying the selected AH temperature to all potato varieties, results showed that Kennebec had the highest TPC values, followed by Agria and Neiker, which had the highest antioxidant activity values measured by ABTS method. Although TPC contribution to the antioxidant activity is well established, Riciputi et al. (2018) reported the presence of different phenolic compounds in different potato peels, contributing unevenly to the antioxidant activity of these matrices.

Several authors reported a relationship between the potato peel colour characteristics and the TPC content and antioxidant capacity, with red- or purple-peel potatoes containing higher amounts of phenolic compounds compared with yellow-peel cultivars (Jeddou et al., 2018; Perla, Holm, & Jayanty, 2012; Tierno et al., 2016). In the present study, Neiker, the red peel variety showed lower TPC values than yellow-peel potatoes (Kennebec and Agria). It is worth mentioning that discarded potatoes stored at room temperature were used, which could account for the differences observed, as storage time and temperature greatly affects xanthophyll and phenolic content, as well as antioxidant capacity (Blessington et al., 2015; Galani et al., 2017).

In a previous study, the authors (Torres et al., 2020) reported that Agria potato peel also presented lower Na contents and much higher Fe and 60% more protein contents than Kennebec and Neiker varieties, which could contribute to a nutritionally rich liquid fraction than the other potato varieties.

Taking these results into account, Agria was the selected potato variety to be tested in the production of pasta product.

3.2. Pasta cooking quality

The results for the pasta quality performance upon cooking are shown in Fig. 1.

Functional ingredients introduced into the pasta formulation, especially proteins or fibre, are known to significantly influence its properties (Fradinho, Raymundo, et al., 2019; Fradique et al., 2013). Control and pasta produced with potato peel extract (PPE) present distinct water absorption and swelling power. As reported in an earlier study (Torres et al., 2020), Agria potato peel showed around 22% protein content. During autohydrolysis procedure, proteins are cleaved into water soluble peptides which are then extracted to the liquor (Castro-Puyana, Herrero, Mendiola, & Ibáñez, 2013), thus contributing for the increase of the hydration properties of pasta. Moreover, PPE

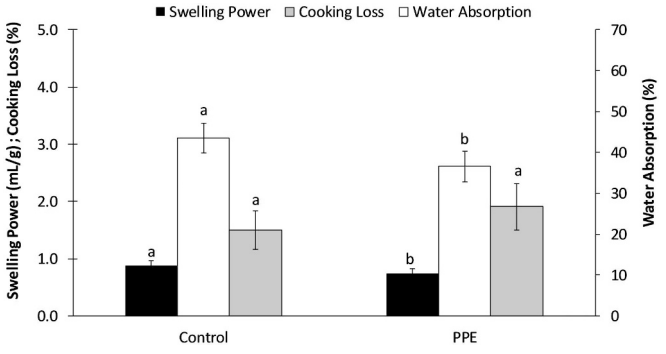


Fig. 1. Cooking quality parameters of pasta prepared with potato peel extract (PPE), and control (without extract). Data are presented as mean ± standard deviation. Different letters in the same parameter correspond to significant differences ($p < 0.05$, two sample *t*-test).

pasta showed a higher protein content than the control (Table 3) reinforcing the previous statement.

Regarding cooking loss, although the replacement of wheat flour by other non-gluten flour dilutes the strength of pasta network that holds the starch particles together, our CL results were lower than the ones reported by other authors for durum wheat pasta (e.g. Bonomi et al., 2012) stating the quality of the developed pastas. Ferreira et al. (2016) also obtained low CL values for GF pasta with potato starch, rice flour, sorghum, eggs, oil, water, therefore a much more complex formulation than the one porposed in this study. Furthermore, this simpler formula follows consumer demands for clean label sustainable foods with by-products from the food industry.

3.3. Colour stability upon cooking

Colour stability is an important attribute for the development of colourful food products. In Table 2 the results of colour parameters obtained in raw and cooked pastas are shown.

As expected, the addition of potato peel extract (PPE) changed the colour of the GF pasta, which had a pale brown colour. After thermal processing (cooking – boiling in water) both pastas revealed some change in sample colour, meaning a pigment loss (leaching) to the cooking water. All ΔE* values showed much higher values than 5, which means that the colour difference between the raw and cooked pastas is visible to the human eye (Castellar, Obón, & Fernández-López, 2006). Since the main pigments responsible for potato colour are phenolic compounds, which have hydrophilic character, this explains pigment leaching into the cooking water. In addition, colour changes during heat treatment may be associated to the phenol oxidation reaction as a result of polyphenol oxidase enzyme activity (Parveen, Threadgill, Moorby, & Winters, 2010).

3.4. Nutritional composition of pasta

In Table 3 the nutritional composition of raw and cooked pasta samples is presented.

In terms of nutritional composition, the addition of potato peel extract led to a significant increase ($p < 0.001$) of the protein and ash contents of pasta, which is maintained after cooking. Although raw PPE also showed a lower lipid content than the Control, both pastas presented similar values after cooking, meaning that free fatty acids were probably leached into the cooking water.

Upon cooking, ash content of Control remained constant, but in PPE there was a 37% loss, although still higher than the Control. This is interesting and must be due to the entrapment of potato minerals in the pasta matrix. In terms of energy value, the cooked enriched pasta aports 3% less energy value than of the Control (EV_{PPE} = 390.9 kcal/100 g; $EV_{Control}$ = 403.2 kcal/100 g).

Although potato peel presents around 22% of non-starch polysaccharides (Liang & McDonald, 2014) that could increase the dietary fibre content, these compounds are not water-soluble and therefore extracted to a great extent by autohydrolysis procedure. The insoluble fibre observed in PPE is most probably due to fibre contribution from the other pasta ingredients, namely Psyllium husk and rice (Raymundo, Fradinho, & Nunes, 2014).

Nutrition claims are assessed on the basis of the intake of the specific nutrient in the ready-to-eat food product. All cooked pastas showed < 0.5 g lipids/100 g, consequently they can withstand a fat-free claim (Regulation (EC) No. 1924/2006 (European Commission, 2006) amended by regulation (EU) no. 1047/2012) (European Commission, 2012), falling within the current trend for healthy foods.

Looking more closely at the mineral composition of cooked pastas, a significant improvement ($p < 0.001$) of major (Na, K, Mg and P) and trace minerals (Cu and I) was obtained. It is noteworthy that the increase in copper (10.4% to 39.7% RDA) and iodine (15.0% to 126.5%) contents represent > 15% of the recommended daily allowance (RDA)

Table 2
Colour parameters (L*, a*, b*, $\Delta E^*_{raw\text{-}cooked}$ and $\Delta E^*_{Control\text{-}PPE}$) of raw and cooked pastas.

	Control		PPE		$\Delta E^*_{Control\text{-}PPE}$	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
L*	82.51 ± 0.40^a	66.69 ± 0.40^c	69.42 ± 0.49^b	50.65 ± 0.65^d	20.7	33.5
a*	1.08 ± 0.08^c	−0.14 ± 0.07^d	6.51 ± 0.17^b	7.58 ± 0.21^a		
b*	8.92 ± 0.21^c	5.92 ± 0.37^d	25.29 ± 0.41^a	16.82 ± 0.59^b		
$\Delta E^*_{raw\text{-}cooked}$	16.1		20.6			

Data are presented as mean ± standard deviation. Different letters in the same parameter correspond to significant differences ($p < 0.001$, one-way ANOVA, *post-hoc* Tukey test). $\Delta E^*_{raw\text{-}cooked}$ and $\Delta E^*_{Control\text{-}PPE}$ calculated using raw pasta and control pasta as reference, respectively.

according to Regulation (EC) 1169/2011 (European Commission, 2011). These values are much higher than the values reported by Orecchio et al. (2014) for commercial rice noodles and pasta, and in case of Cu it has functions on the cardiovascular integrity, lung elasticity, neovascularization, neuroendocrine function, and iron metabolism (Arredondo & Núñez, 2005), and I in regulating thyroid disease as celiac patients are more susceptible to, than non-celiacs (Torres et al., 2015).

Raw PPE pasta showed much higher Fe content than the Control, due to its content in Agria peel (485 mg/100 g) as reported by Torres et al. (2020). However, upon cooking some of this mineral is leached to the cooking water, so that Control and PPE pastas showed similar Fe contents.

These results showed that the potato peel extract addition can be used to enhance the nutritional value of GF pasta, increasing the amount of protein and minerals profile.

3.5. Phytochemical analysis in the pasta

The results of total phenolic content (TPC) and in vitro antioxidant activity (AA) of pastas, performed by DPPH and ABTS methods are summarised in Fig. 2. As pasta is consumed after cooking, the impact of thermal processing should be assessed, as it induces great changes in texture, molecule structure, nutritional content and availability (Carcea, Narducci, Turfani, & Giannini, 2017).

For both raw and cooked pastas, the TPC of potato peel enriched pasta (PPE) is around 9 times higher than of the Control. This aspect is relevant and raises the interest on the use of potato peel extracts in pasta. In addition, although a detailed sensory analysis was not carried out, in the preliminary tests of the PPE pasta, there was a very pleasant aroma and flavour (coffee-like). The presence of free-form phenolic compounds (such as chlorogenic acid and caffeic acid) has been reported in potato (e.g. Friedman et al., 2017) and already obtained using environmentally friendly extraction technologies (Alves Filho, Sousa, Rodrigues, de Brito, & Fernandes, 2020). Moreover, during potato storage at room temperature chlorogenic acid is transformed into caffeic acid (Wijngaard, Ballay, & Brunton, 2012). However, further studies on the flavours released could be performed to enlighten this matter.

3.6. Texture analysis of pasta

The addition of potato peel extract had no effect on most of the texture properties (Table 4), except for an increase in raw pasta adhesiveness. This result was lower than the observed for GF pasta enriched with *Laminaria ochroleuca* autohydrolysis extract probably due to the lower content of insoluble fibre of PPE (Fradinho, Raymundo, et al., 2019), as fibre absorbs water thus contributing to the adhesiveness of pasta (Bouasla, Wojtowicz, & Zidoune, 2017). These results are also advantageous in terms of the final product, as the consumer will find characteristics of texture and technological aptitude very similar to traditional pasta.

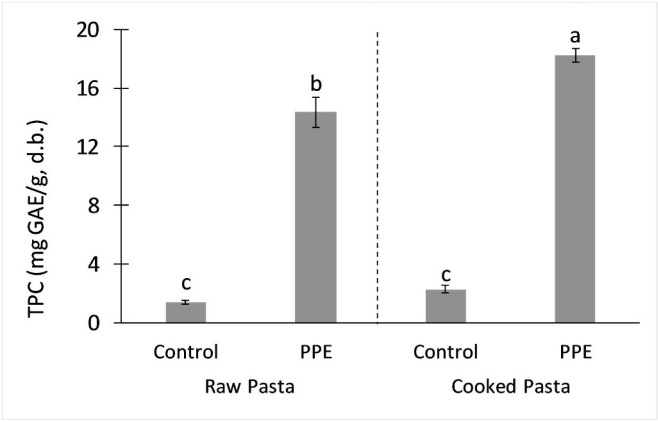
3.7. Rheology characterization of pasta samples

The results from the small amplitude dynamic rheology measurements of the raw and cooked pastas are expressed in terms of storage (G′) and loss (G″) moduli (Fig. 3).

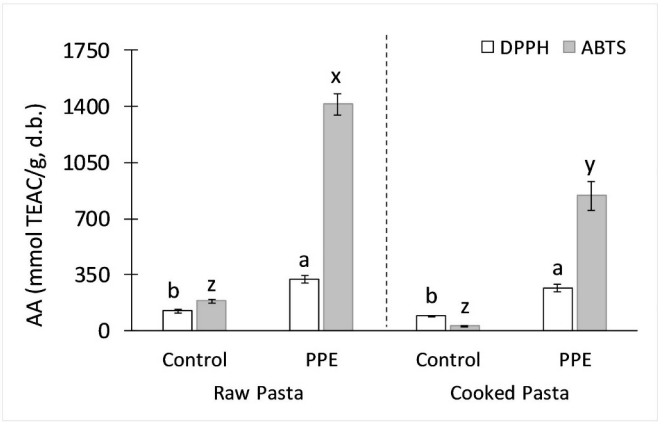
Table 3
Nutritional composition of raw and cooked pasta with potato peel extract (PPE) and Control (without extract).

			Control		PPE	
			Raw	Cooked	Raw	Cooked
	Moisture	g/100 g	50.3 ± 0.2^b	65.4 ± 1.4^a	48.9 ± 0.1^b	64.2 ± 1.2^a
Fibre	Ash	g/100 g, d.b.	0.46 ± 0.01^c	0.43 ± 0.03^c	0.87 ± 0.02^a	0.55 ± 0.02^b
	Lipids		2.17 ± 0.22^a	0.64 ± 0.18^b	0.83 ± 0.27^b	0.83 ± 0.13^b
	Protein		7.66 ± 0.15^b	7.72 ± 0.01^b	8.24 ± 0.13^a	8.46 ± 0.31^a
	Insoluble			4.83 ± 0.39^a		4.04 ± 0.11^b
	Soluble			0.94 ± 0.25^a		1.47 ± 0.50^a
	Total			6.05 ± 0.69^a		5.51 ± 0.57^a
Minerals	Na	mg/100 g, d.b	74.11 ± 2.79^{b,c}	70.00 ± 3.28^c	88.26 ± 2.34^a	82.84 ± 4.94^{a,b}
	K		325.26 ± 3.28^c	272.37 ± 4.36^d	531.86 ± 1.88^a	400.67 ± 3.26^b
	Ca		7.35 ± 1.61^a	9.92 ± 1.76^a	10.17 ± 0.54^a	9.26 ± 1.08^a
	Mg		28.26 ± 0.22^c	27.58 ± 0.48^c	36.24 ± 0.35^a	30.89 ± 0.10^b
	P		115.78 ± 2.33^b	109.54 ± 0.65^c	129.58 ± 3.05^a	118.18 ± 1.45^b
	S		109.00 ± 4.15^{a,b}	104.70 ± 1.75^b	117.36 ± 5.25^a	112.64 ± 2.91^{a,b}
	Fe		0.68 ± 0.35^b	1.16 ± 0.08^b	2.67 ± 0.42^a	1.35 ± 0.36^b
	Cu		0.27 ± 0.00^b	0.26 ± 0.14^b	0.27 ± 0.10^b	1.11 ± 0.28^a
	Zn		1.06 ± 0.04^b	1.32 ± 0.12^{a,b}	1.33 ± 0.03^{a,b}	1.65 ± 0.36^a
	Mn		0.75 ± 0.12^a	0.77 ± 0.14^a	0.76 ± 0.06^a	0.77 ± 0.04^a
	I		0.10 ± 0.00^c	0.07 ± 0.00^c	0.43 ± 0.02^b	0.53 ± 0.03^a

Data are presented as mean ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.001$, one-way ANOVA, post-hoc Tukey test and $p < 0.05$, two sample *t*-test).



a



b

Fig. 2. Total phenolic content (a) and Antioxidant Activity (b) of raw and cooked pastas: with Potato peel extract (PPE) and control (without extract). DPPH: α,α-diphenyl-b-picrylhydrazyl; ABTS: 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate); TPC: total phenolic content; GAE: gallic acid equivalent; AA: antioxidant activity; TEAC: Trolox equivalent antioxidant capacity. Different letters in the same parameter correspond to significant differences ($p < 0.001$, one-way ANOVA, *post-hoc* Tukey test).

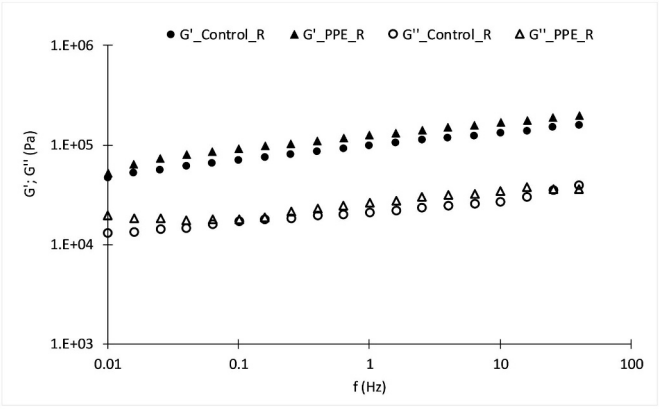
Table 4
Texture of raw and cooked pasta with potato peel extract (PPE) and Control (without extract).

		Control	PPE
Raw pasta	Firmness (N)	3.24 ± 0.54[a]	3.26 ± 0.41[a]
	Adhesiveness (−N.s)	0.24 ± 0.09[a]	0.38 ± 0.10[b]
	Cohesiveness	0.47 ± 0.02[a]	0.45 ± 0.02[a]
Cooked pasta	Firmness (N)	2.76 ± 0.29[a]	2.49 ± 0.20[a]
	Stickiness (N)	1.51 ± 0.44[a]	1.23 ± 0.45[a]
	Rmax (N)	0.58 ± 0.10[a]	0.64 ± 0.08[a]
	ERmax (mm)	4.61 ± 1.40[a]	5.08 ± 0.93[a]

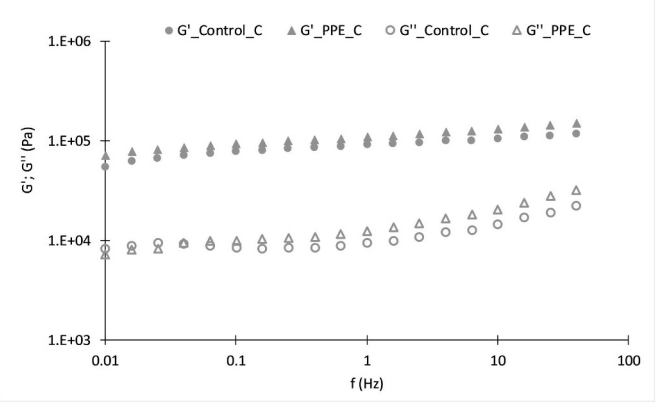
Data are presented as mean ± standard deviation. Letters in the same row correspond to significant differences ($p < 0.05$, two sample *t*-test).

For both raw and cooked pastas the storage modulus values (G′) are higher than those of the loss modulus (G″), which reveals the more elastic nature of the structure of the studied samples.

Raw pastas showed a rheology dependent on the frequency, but upon cooking, there was a decrease in both viscoelastic moduli accompanied by an increase in G′ and G″ distance, meaning a softened matrix but with a reinforcement in the pasta structure. After thermal



a



b

Fig. 3. Mechanical spectra of raw (a) and cooked (b) GF pastas: with Potato Peel Extract (PPE) and control (without extract). Closed symbols - storage modulus, G′; open symbols - loss modulus, G″.

treatment pasta also showed a less dependence of G′ with the frequency and a minimum in G″, which indicated a second degree of structuring due to the entanglement of the biomolecules that comprise the food matrix (Ferry, 1980). This behaviour is consistent with the one previously observed by the authors regarding GF pasta enriched with edible brown seaweed (Fradinho, Raymundo, et al., 2019).

4. Conclusions

Low-sized or irregular shape discarded potatoes have a great potential to be valued back into the food chain. Subcritical water extraction (220 °C) was an efficient eco-friendly technology that allowed the recovery of bioactive fractions from the potato peel. Potato peel extract revealed to be an attractive ingredient to improve the nutritional value of gluten-free pasta, increasing its mineral content, total phenolic content and antioxidant activity, without affecting the mechanical properties of the product.

Credit author statement

Patrícia Fradinho: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original draft preparation; António Oliveira: Investigation; Herminia Domínguez: Funding acquisition, Supervision, Writing- Reviewing and Editing; María Dolores Torres: Investigation, Writing- Reviewing and Editing; Isabel Sousa: Writing- Reviewing and Editing, Funding acquisition; Anabela Raymundo: Conceptualization, Supervision, Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

NP 518 (1986). *Cereais e Leguminosas – Determinação do teor de cinza. Processo por incineração a 550 °C*Lisboa, Portugal: Instituto Português da Qualidade.

NP 4168 (1991). *Cereais e Derivados—Determinação do Teor de Matéria Gorda Total.* Lisboa, Portugal: Instituto Português da Qualidade.

FAO (2003). *Food energy—Methods of analysis and conversion factors.* Rome, Italy: Food and Agriculture Organization of the United Nations93.

ISO 20483 (2006). *Cereals and pulses—Determination of the nitrogen content and calculation of the crude protein content—Kjeldahl method.* Geneva, Switzerland: International Organization for Standardization.

AACC (1999a). *Method 66-50.01 – Pasta and noodle cooking quality—Firmness. Approved methods of analysis* (11th ed.). St. Paul, MN, USA: AACC International. https://doi.org/10.1094/AACCIntMethod-66-50.01.

AACC (Ed.). (1999). *Method 44-15.02 – Moisture: Air-oven methods. Approved methods of analysis*(11th ed.). St. Paul, MN, USA: AACC International. https://doi.org/10.1094/AACCIntMethod-44-15.02.

Akyol, H., Riciputi, Y., Capanoglu, E., Caboni, M. F., & Verardo, V. (2016). Phenolic compounds in the potato and its byproducts: An overview. *International Journal of Molecular Sciences, 17*(6), 835. https://doi.org/10.3390/ijms17060835.

Alves Filho, E. G., Sousa, V. M., Rodrigues, S., de Brito, E. S., & Fernandes, F. A. N. (2020). Green ultrasound-assisted extraction of chlorogenic acids from sweet potato peels and sonochemical hydrolysis of caffeoylquinic acids derivatives. *Ultrasonics Sonochemistry, 63*, 104911. https://doi.org/10.1016/j.ultsonch.2019.104911.

AOAC (Ed.). (1998). *Method 991.43 - Total, soluble, and insoluble dietary fiber in foods. AOAC official methods of analysis*(16th ed.). Gaithersburg, MD, USA: AOAC International.

Arredondo, M., & Núñez, M. T. (2005). Iron and copper metabolism. *Molecular Aspects of Medicine, 26*(4–5), 313–327. https://doi.org/10.1016/j.mam.2005.07.010.

Ballesteros, L. F., Ramirez, M. J., Orrego, C. E., Teixeira, J. A., & Mussatto, S. I. (2017). Optimization of autohydrolysis conditions to extract antioxidant phenolic compounds from spent coffee grounds. *Journal of Food Engineering, 199*, 1–8. https://doi.org/10.1016/j.jfoodeng.2016.11.014.

Blessington, T., Scheuring, D. C., Nzaramba, M. N., Hale, A. L., Reddivari, L., Vestal, T. A., ... Miller, J. C. (2015). The use of low-dose electron-beam irradiation and storage conditions for sprout control and their effects on xanthophylls, antioxidant capacity, and phenolics in the potato cultivar Atlantic. *American Journal of Potato Research, 92*, 609–618. https://doi.org/10.1007/s12230-015-9474-4.

Bonomi, F., D'Egidio, M. G., Iametti, S., Marengo, M., Marti, A., Pagani, M. A., & Ragg, E. M. (2012). Structure–quality relationship in commercial pasta: A molecular glimpse. *Food Chemistry, 135*, 348–355. https://doi.org/10.1016/j.foodchem.2012.05.026.

Bouasla, A., Wojtowicz, A., & Zidoune, M. N. (2017). Gluten-free precooked rice pasta enriched with legumes flours: Physical properties, texture, sensory attributes and microstructure. *LWT - Food Science & Technology, 75*, 569–577. https://doi.org/10.1016/j.lwt.2016.10.005.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Carcea, M., Narducci, V., Turfani, V., & Giannini, V. (2017). Polyphenols in raw and cooked cereals/pseudocereals/legume pasta and couscous. *Foods, 6*(9), 80. https://doi.org/10.3390/foods6090080.

Castellar, M. R., Obón, J. M., & Fernández-López, J. A. (2006). The isolation and properties of a concentrated red-purple betacyanin food colorant from *Opuntia stricta* fruits. *Journal of the Science of Food and Agriculture, 86*, 122–128. https://doi.org/10.1002/jsfa.2285.

Castro-Puyana, M., Herrero, M., Mendiola, J.Á., & Ibáñez, E. (2013). Subcritical water extraction of bioactive components from algae. In H. Domínguez (Ed.). *Functional ingredients from algae for foods and nutraceuticals* (pp. 534–560). Cambridge: Woodhead Publishing.

Díaz-Reinoso, B., González-Muñoz, M. J., & Domínguez, H. (2017). Introduction. In H. Domínguez, & M. J. González-Muñoz (Eds.). *Water extraction of bioactive compounds: From plants to drug development* (pp. 1–50). Amsterdam, the Netherlands: Elsevier. https://doi.org/10.1016/B978-0-12-809380-1.00001-2.

European Commission (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1924&from=PT.

European Commission (2011). *Regulation (EC) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers.* https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169&from=pt.

European Commission (2012). *(EU) No 1047/2012 of 8 November 2012 amending Regulation (EC) No 1924/2006 with regard to the list of nutrition claims.* https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R1047&from=PT.

FAO (2008). International year of the potato. URL http://www.fao.org/potato-2008/en/index.html.

FAOSTAT (2019). Statistics division of the Food and Agriculture Organization of the United Nations. URL: http://www.fao.org/faostat/en/#data/QC/visualize.

Ferreira, S. M. R., de Mello, A. P., dos Anjos, M. C. R., Krüger, C. C. H., Azoubel, P. M., & Alves, M. A. O. (2016). Utilization of sorghum, rice, corn flours with potato starch for the preparation of gluten-free pasta. *Food Chemistry, 191*, 147–151. https://doi.org/10.1016/j.foodchem.2015.04.085.

Ferry, J. D. (1980). *Viscoelastic properties of polymers* (3rd ed.). Hoboken, NJ, USA: John Wiley & Sons Inc672.

Flórez-Fernández, N., Torres, M. D., González-Muñoz, M. J., & Domínguez, H. (2019). Recovery of bioactive and gelling extracts from edible brown seaweed *Laminaria ochroleuca* by non-isothermal autohydrolysis. *Food Chemistry, 277*, 353–361. https://doi.org/10.1016/j.foodchem.2018.10.096.

Fradinho, P., Flórez-Fernández, N., Sousa, I., Raymundo, A., Domínguez, H., & Torres, M. D. (2020). Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-019-01958-8.

Fradinho, P., Raymundo, A., Sousa, I., Domínguez, H., & Torres, M. D. (2019). Edible brown seaweed in gluten-free pasta: Technological and nutritional evaluation. *Foods, 8*(12), 622. https://doi.org/10.3390/foods8120622.

Fradinho, P., Soares, R., Niccolai, A., Sousa, I., & Raymundo, A. (2020). *Psyllium* husk gel to reinforce structure of gluten-free pasta? *LWT - Food Science and Technology* LWT-D-20-00819, Submitted).

Fradinho, P., Sousa, I., & Raymundo, A. (2019). Functional and thermorheological properties of rice flour gels for gluten-free pasta applications. *International Journal of Food Science and Technology, 54*(4), 1109–1120. https://doi.org/10.1111/ijfs.14001.

Fradique, M., Batista, A. P., Nunes, C. M., Gouveia, L., Bandarra, N. M., & Raymundo, A. (2013). *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source. *LWT - Food Science & Technology, 50*, 312–319. https://doi.org/10.1016/j.lwt.2012.05.006.

Friedman, M., Kozukue, N., Kim, H. J., Choi, S. H., & Mizunod, M. (2017). Glycoalkaloid, phenolic, and flavonoid content and antioxidative activities of conventional non-organic and organic potato peel powders from commercial gold, red, and Russet potatoes. *Journal of Food Composition and Analysis, 62*, 69–75. https://doi.org/10.1016/j.jfca.2017.04.019.

Galani, J. H. Y., Mankad, P. M., Shah, A. K., Patel, N. J., Acharya, R. R., & Talati, J. G. (2017). Effect of storage temperature on vitamin C, total phenolics, UPLC phenolic acid profile and antioxidant capacity of eleven potato (*Solanum tuberosum*) varieties. *Horticultural Plant Journal, 3*(2), 73–89. https://doi.org/10.1016/j.hpj.2017.07.004.

Gientka, I., Aleksandrzak-Piekarczyk, T., Bzducha-Wróbel, A., Synowiec, A., & Błazejak, S. (2019). Deproteinated potato wastewater as a sustainable nitrogen source in Trichosporon domesticum yeast lipids biosynthesis—A concept of valorization of wastewater from starch industry. *Potato Research, 62*, 221–237. https://doi.org/10.1007/s11540-018-9408-x.

Huamán-Leandro, L. R., González-Muñoz, M. J., Fernández-De-Ana, C., Rodríguez-Blanco, A., Torres, M. D., & Domínguez, H. (2020). Autohydrolysis of *Lentinus edodes* for obtaining extracts with antiradical properties. *Foods, 9*(1), 74. https://doi.org/10.3390/foods9010074.

Iriondo-DeHond, A., García, N. A., Fernandez-Gomez, B., Guisantes-Batan, E., Escobar, F. V., Blanch, G. P., ... del Castillo, M. D. (2019). Validation of coffee by-products as novel food ingredients. *Innovative Food Science and Emerging Technologies, 51*, 194–204. https://doi.org/10.1016/j.ifset.2018.06.010.

Jeddou, K. B., Bouaziz, F., Helbert, C. B., Nouri-Ellouz, O., Maktouf, S., Ellouz-Chaabouni, S., & Ellouz-Ghorbel, R. (2018). Structural, functional, and biological properties of potato peel oligosaccharides. *International Journal of Biological Macromolecules, 112*, 1146–1155. https://doi.org/10.1016/j.ijbiomac.2018.02.004.

Lee, S. C., Rodriguez, F., & Storey, M. (1995). Determination of soluble and insoluble dietary fiber in Psyllium-containing cereal products. *Journal of AOAC International, 78*(3), 724–729. https://www.ncbi.nlm.nih.gov/pubmed/7756888.

Liang, S., & McDonald, A. G. (2014). *Chemical and thermal characterization of potato peel waste and its*.

López-Hortas, L., Gannon, L., Moreira, R., Chenlo, F., Domínguez, H., & Torres, M. D. (2018). Microwave hydrodiffusion and gravity (MHG) processing of *Laminaria ochroleuca* brown seaweed. *Journal of Cleaner Production, 197*, 1108–1116. https://doi.org/10.1016/j.jclepro.2018.06.274.

Nara, K., Miyoshi, T., Honma, T., & Koga, H. (2006). Antioxidative activity of bound-form phenolics in potato peel. *Bioscience, Biotechnology, and Biochemistry, 70*(6), 1489–1491. https://doi.org/10.1271/bbb.50552.

Orecchio, S., Amorello, D., Raso, M., Barreca, S., Lino, C., & Di Gaudio, F. (2014). Determination of trace elements in gluten-free food for celiac people by ICP-MS. *Microchemical Journal, 116*, 163–172. https://doi.org/10.1016/j.microc.2014.04.011.

Pal, K. G., & Suresh, P. V. (2016). Sustainable valorisation of seafood by-products: Recovery of collagen and development of collagen-based novel functional food ingredients. *Innovative Food Science and Emerging Technologies, 37*, 201–215. https://doi.org/10.1016/j.ifset.2016.03.015.

Parveen, I., Threadgill, M. D., Moorby, J. M., & Winters, A. (2010). Oxidative phenols in forage crops containing polyphenol oxidase enzymes. *Journal of Agriculture and Food Chemistry, 58*, 1371–1382. https://doi.org/10.1021/jf9024294.

Perla, V., Holm, D. G., & Jayanty, S. S. (2012). Effects of cooking methods on polyphenols, pigments and antioxidant activity in potato tubers. *LWT - Food Science and Technology, 45*, 161–171. https://doi.org/10.1016/j.lwt.2011.08.005.

Plaza, M., Amigo-Benavent, M., del Castillo, M. D., Ibáñez, E., & Herrero, M. (2010). Facts about the formation of new antioxidants in natural samples after subcritical water extraction. *Food Research International, 43*, 2341–2348. https://doi.org/10.1016/j.foodres.2010.07.036.

Priedniece, V., Spalvins, K., Ivanovs, K., Pubule, J., & Blumberga, D. (2017). Bioproducts from potatoes - A review. *Environmental Climate Technology, 21*, 18–27. https://doi.org/10.1515/rtuect-2017-0013.

Rajauria, G., Jaiswal, A. K., Abu-Ghannam, N., & Gupta, S. (2010). Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds. *International Journal of Food Science & Technology, 45*, 2485–2493. https://doi.org/10.1111/j.1365-2621.2010.02449.x.

Raymundo, A., Fradinho, P., & Nunes, M. C. (2014). Effect of Psyllium fibre content on the textural and rheological characteristics of biscuit and biscuit dough. *Bioactive Carbohydrates and Dietary Fibre, 3*, 96–105. https://doi.org/10.1016/j.bcdf.2014.03.001.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Riciputi, Y., Diaz-de-Cerio, E., Akyol, H., Capanoglu, E., Cerretani, L., Caboni, M. F., & Verardo, V. (2018). Establishment of ultrasound-assisted extraction of phenolic compounds from industrial potato by-products using response surface methodology. *Food Chemistry, 269*, 258–263. https://doi.org/10.1016/j.foodchem.2018.06.154.

Sant'Anna, V., Christiano, F. D. P., Marczak, L. D. F., Tessaro, I. C., & Thys, R. C. S. (2014). The effect of the incorporation of grape marc powder in fettuccini pasta properties. *LWT - Food Science & Technology, 58*, 497–501. https://doi.org/10.1016/j.lwt.2014.04.008.

Singh, P. P., & Saldaña, M. D. (2011). Subcritical water extraction of phenolic compounds from potato peel. *Food Research International, 44*, 2452–2458. https://doi.org/10.1111/ijfs.14361.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Tierno, R., López, A., Riga, P., Arazuri, S., Jarén, C., Benedicto, L., & Ruiz de Galarreta, J. I. (2016). Phytochemicals determination and classification in purple and red fleshed potato tubers by analytical methods and near infrared spectroscopy. *Journal of the Science of Food and Agriculture, 96*, 1888–1899. https://doi.org/10.1002/jsfa.7294.

Torres, M. D., Chenlo, F., & Moreira, R. (2018). Rheological effect of gelatinisation using different temperature-time conditions on potato starch dispersions: Mechanical characterisation of the obtained gels. *Food Bioprocess Technology, 11*, 132–140. https://doi.org/10.1007/s11947-017- 2000-6.

Torres, M. D., Fradinho, P., Rodríguez, P., Falqué, E., Santos, V., & Domínguez, H. (2020). Biorefinery concept for discarded potatoes: Recovery of starch and bioactive compounds. *Journal of Food Engineering, 275*, 109886. https://doi.org/10.1016/j.jfoodeng.2019.109886.

Torres, M. I., Palomeque, T., & Lorite, P. (2015). Celiac disease and other autoimmune disorders. In K. Chatzidionysiou (Vol. Ed.), *Autoimmunity—Pathogenesis, clinical aspects and therapy of specific autoimmune diseases. 6. Autoimmunity—Pathogenesis, clinical aspects and therapy of specific autoimmune diseases* (pp. 131–151). London, UK: IntechOpen.

Wijngaard, H., Ballay, M., & Brunton, N. (2012). The optimisation of extraction of antioxidants from potato peel by pressurised liquids. *Food Chemistry, 133*(4), 1123–1130. https://doi.org/10.1016/j.foodchem.2011.01.136.

Chapter 6. Overall conclusions and future perspectives

6.1. Overall conclusions

Following consumer trends, both researchers and the food industry are focusing on the development of healthy food products with clean labels to fulfil consumer expectations and needs for more natural foods, made from ingredients that are recognized, sustainable, locally produced and authentic.

In this context, this thesis proposed the alignment of these trends with the development of gluten-free fresh pastas based on rice flour, enriched with bioactive compounds.

Rice flour, the raw material for the proposed products, is an industrial by-product from broken grains resulting from the dehusking and polishing of rice kernels. For the preparation of gluten free pasta rice flour from two rice varieties was used: Guiana and Ariete. Flour from both rice varieties showed good gelling abilities for food applications, such as gluten-free pasta (C2). This GF pasta was successfully prepared with 50% pre gelatinised rice flour (20% rice flour) + 50% rice flour with good overall cooking quality and texture parameters. The pre-gelatinisation of rice flour proved to be a technological process suited for the starch modification needed to build up structure for the gluten-free pasta.

The use of *Psyllium* husk, a natural source of biopolymers, that forms gel at room temperature was successfully employed in the development of GF fresh pasta (C3). This fibre addition made possible to eliminate the starch pre-gelatinization step. This is a significant point of innovation which can have a relevant impact on industrial processing of GF pasta in terms of time and energy expenditure. Furthermore, the developed GF pasta: 50% rice flour + 50% *Psyllium* gel (4% *Psyllium* husk) showed higher cooking and textural quality properties with decreasing carbohydrate *in vitro* digestibility compared to the control. In addition, the use of *Psyllium* husk allowed the development of a *clean label* product, composed only by water, rice flour and *Psyllium* husk, in line with current consumer trends.

In line with bioeconomy targets of adding value to by-products, the use of eco-friendly technologies for the extraction of functional components also contributes to the sustainability of the food industry. Subcritical water extraction (autohydrolysis) is a *green* technology that is a green technology that has been successfully applied to value under-exploited resources such as seaweed (C4.2) and by-products of the food industry like potato peel (C5.3). This process enabled the recovery of bioactive-rich extracts of both materials through depolymerisation and solubilisation of components with proven health benefits. The combination of autohydrolysis (AH) procedure with ultrasounds resulted in relevant changes in rheology and texture properties of *Laminaria* purée-like mixtures, expanding the pallet of possible applications. Taking advantage of the gelling ability of Psyllium husk, food gels were developed by combining this natural resource with the brown seaweed (C4.3). A positive interaction between both materials was found, reinforcing the viscoelastic behaviour of the obtained gels.

Corresponding to the healthy food demand and to fulfil some of the nutritional unbalances of the celiac population, three studies were designed to enrich the gluten-free pasta developed with bioactive compounds from *Laminaria ochroleuca* (C5.1), *Arthrospira platensis* (C5.2) and potato peel (C5.3). *Laminaria ochroleuca* showed promising potential, to be valued either in full or using its liquid extract, for pasta applications in terms of fibre and mineral contents with interesting mechanical properties. Also, GF pasta enriched with this brown seaweed could bear nutrition claims for *source of fibre* and *fat-free*.

The use of *Arthrospira platensis* biomasses (C5.2) resulted in GF pastas with attractive and innovative appearance, enhanced nutritional quality (TPC, chlorophylls and carotenoids) and antioxidant capacity, without affecting its cooking and texture quality properties, with a favourable sensory evaluation.

The different drying methods (lyophilization and spray-drying) applied to *A. platensis* biomasses had an impact on the bioactive compounds and on the *in vitro* digestibility of the GF pastas.

Potato peel (C5.3) use was also in line with the main objectives of this thesis, i.e. the recovery and valorisation of by-products of the food industry. GF pasta enriched with potato peel AH extract showed higher extensibility properties than the control, coupled with attractive colour, high total phenolic content and antioxidant activity.

6.2. Future perspectives

This thesis presents an approach for the development of gluten-free fresh pasta enriched with bioactive compounds. Due to the intrinsic characteristic of a scientific study, the products proposed may be further enhanced.

- The pastas developed are fresh products, designed to be produced and consumed immediately, or preserved at refrigerator temperatures. Packaging studies should be performed, targeting the development of an eco-friendly package, to ensure the proper conservation of the products without neglecting sustainability issues. Consequently, shelf life assessment of the products should be performed.

- *Psyllium* husk used in this work is a United States commercial product using *Psyllium* seeds from India (C3). Although this product is available in the Portuguese market, the use of *Psyllium* from Madeira island (Portugal) should be considered in terms of sustainability (economic and environmental). This material is known by the local population, yet it is an under-exploited resource, as it is not industrially produced.

- Only a fraction of the nutrients is absorbed by the human body, effectively contributing to the individual's health. Studies on the bioavailability of the interesting compounds, namely polyphenols,

minerals, and fibre, should be undertaken to evaluate the real functionally of the developed products. Also, to fully assess the nutritional quality of the gluten-free pastas, the glycemic index of the developed products should be assessed.

- The GF pastas developed were also designed for celiac consumers. Therefore, the industrial production should take place in a gluten-free environment, and the detection of gluten should be performed to assure that the products comply with the regulations (Regulation (EU) No 828/2014). This population has a different sense of taste than of non-celiac, therefore a sensory analysis should be performed with a consumer panel of celiac consumers, in collaboration with APC (Celiac Portuguese Association) who also can validate the products as gluten-free through its stamp.

- From the industrial point of view, it would be important to consider the steps required to scale-up the production process and take this product to a commercial level. In this sense, a cost/benefit analysis should be performed as well as the establishment of collaborations with industrial partners due to the gluten-free nature of the products.